Cumulative Dissertation

Eco-physiological Consequences of Fluctuating Light on Phytoplankton

for the degree of Dr. rer. nat.
Ecology

by Alexis Guislain

Submitted to
Faculty of Science,
Institute of Biochemistry and Biology,
University of Potsdam

First supervisor:
Prof. Dr. Ursula Gaedke
Second supervisor:
Dr. Jan Köhler

Evaluation:
Prof. Dr. Ursula Gaedke
Prof. Dr. Brigitte Nixdorf
Prof. Dr. Hendrik Schubert

Potsdam, November 2019
Disputation: 01 April 2020

Published online in the
Institutional Repository of the University of Potsdam:
https://doi.org/10.25932/publishup-46927
https://nbn-resolving.org/urn:nbn:de:kobv:517-opus4-469272

Kumulative Dissertation

Eco-physiological Consequences of Fluctuating Light on Phytoplankton

zur Erlangung des akademischen Grades Dr. rer. nat.
in der Wissenschaftsdisziplin "Ökologie"

von Alexis Guislain

Eingereicht an der
Mathematisch-Naturwissenschaftlichen Fakultät,
Institut für Biochemie und Biologie,
Universität Potsdam

Hauptbetreuerin:
Prof. Dr. Ursula Gaedke
Zweitbetreuer:
Dr. Jan Köhler

Begutachtung:
Prof. Dr. Ursula Gaedke
Prof. Dr. Brigitte Nixdorf
Prof. Dr. Hendrik Schubert

Potsdam, November 2019
Disputation: 01 April 2020

Summary

Phytoplankton growth depends not only on the mean intensity but also on the dynamics of the light supply. The nonlinear light-dependency of growth is characterized by a small number of basic parameters: the compensation light intensity $PAR_{comp\mu}$, where production and losses are balanced, the growth efficiency at sub-saturating light α_μ, and the maximum growth rate at saturating light μ_{max}. In surface mixed layers, phytoplankton may rapidly move between high light intensities and almost darkness. Because of the different frequency distribution of light and/or acclimation processes, the light-dependency of growth may differ between constant and fluctuating light. Very few studies measured growth under fluctuating light at a sufficient number of mean light intensities to estimate the parameters of the growth-irradiance relationship. Hence, the influence of light dynamics on μ_{max}, α_μ and $PAR_{comp\mu}$ are still largely unknown. By extension, accurate modelling predictions of phytoplankton development under fluctuating light exposure remain difficult to make. This PhD thesis does not intend to directly extrapolate few experimental results to aquatic systems – but rather improving the mechanistic understanding of the variation of the light-dependency of growth under light fluctuations and effects on phytoplankton development.

In Lake TaiHu and at the Three Gorges Reservoir (China), we incubated phytoplankton communities in bottles placed either at fixed depths or moved vertically through the water column to mimic vertical mixing. Phytoplankton at fixed depths received only the diurnal changes in light (defined as constant light regime), while phytoplankton received rapidly fluctuating light by superimposing the vertical light gradient on the natural sinusoidal diurnal sunlight. The vertically moved samples followed a circular movement with 20 min per revolution, replicating to some extent the full overturn of typical Langmuir cells. Growth, photosynthesis, oxygen production and respiration of communities (at Lake TaiHu) were

measured. To complete these investigations, a physiological experiment was performed in the laboratory on a toxic strain of *Microcystis aeruginosa* (FACBH 1322) incubated under 20 min period fluctuating light. Here, we measured electron transport rates and net oxygen production at a much higher time resolution (single minute timescale).

The present PhD thesis provides evidence for substantial effects of fluctuating light on the eco-physiology of phytoplankton. Both experiments performed under semi-natural conditions in Lake TaiHu and at the Three Gorges Reservoir gave similar results. The significant decline in community growth efficiencies α_μ under fluctuating light was caused for a great share by different frequency distribution of light intensities that shortened the effective daylength for production. The remaining gap in community α_μ was attributed to species-specific photoacclimation mechanisms and to light-dependent respiratory losses. In contrast, community maximal growth rates μ_{max} were similar between incubations at constant and fluctuating light. At daily growth saturating light supply, differences in losses for biosynthesis between the two light regimes were observed. Phytoplankton experiencing constant light suffered photo-inhibition - leading to photosynthesis foregone and additional respiratory costs for photosystems repair. On the contrary, intermittent exposure to low and high light intensities prevented photo-inhibition of mixed algae but forced them to develop alternative light strategy. They better harvested and exploited surface irradiance by enhancing their photosynthesis. In the laboratory, we showed that *Microcystis aeruginosa* increased its oxygen consumption by dark respiration in the light few minutes only after exposure to increasing light intensities. More, we proved that within a simulated Langmuir cell, the net production at saturating light and the compensation light intensity for production at limiting light are positively related. These results are best explained by an accumulation of photosynthetic products at increasing irradiance and mobilization of these fresh resources by rapid enhancement of dark respiration for maintenance and biosynthesis at decreasing irradiance. At the daily timescale, we showed that the enhancement of photosynthesis at high irradiance for biosynthesis of species increased their maintenance respiratory costs at limiting light. Species-specific growth at saturating light μ_{max} and compensation light intensity for growth $PAR_{comp\mu}$ of species incubated in Lake TaiHu were positively related. Because of this species-specific physiological tradeoff, species displayed different light affinities to limiting and saturating light - thereby exhibiting a gleaner-opportunist tradeoff. In Lake TaiHu, we showed that inter-specific differences in light acquisition traits (μ_{max} and $PAR_{comp\mu}$) allowed coexistence of species on a gradient of constant

light while avoiding competitive exclusion. More interestingly we demonstrated for the first time that vertical mixing (inducing fluctuating light supply for phytoplankton) may alter or even reverse the light utilization strategies of species within couple of days. The intra-specific variation in traits under fluctuating light increased the niche space for acclimated species, precluding competitive exclusion.

Overall, this PhD thesis contributes to a better understanding of phytoplankton eco-physiology under fluctuating light supply. This work could enhance the quality of predictions of phytoplankton development under certain weather conditions or climate change scenarios.

Keywords: Lake TaiHu, Three Gorges reservoir, Functional traits, Tradeoff, Fluctuating light, Phytoplankton photoacclimation, Effective daylength, Photosynthesis, Respiration, Niche partitioning, Non-equilibrium coexistence.

Zusammenfassung

Das Wachstum von Phytoplankton hängt ab nicht nur von der mittleren Intensität, sondern auch von der Dynamik des verfügbaren Lichts. Die nicht-lineare Lichtabhängigkeit des Wachstums kann durch drei Parameter beschrieben werden: die Kompensationslichtintensität $PAR_{comp\mu}$, bei der Bruttoproduktion und Verluste gleich sind, die Wachstumseffizienz bei Lichtlimitation α_μ und die maximale Wachstumsrate bei sättigendem Licht μ_{max}. In durchmischten Schichten nahe der Gewässeroberfläche kann das Phytoplankton innerhalb weniger Minuten zwischen Starklicht und nahezu völliger Dunkelheit bewegt werden. Durch die unterschiedliche Häufigkeitsverteilung der Lichtintensitäten und/oder unterschiedliche Anpassungen kann die Lichtabhängigkeit des Wachstums sich bei fluktuierendem Licht von dem bei konstantem Licht unterscheiden. Bislang wurde die Lichtabhängigkeit des Wachstums bei fluktuierendem Licht nur in sehr wenigen Studien für genügend viele Lichtintensitäten gemessen, um die genannten Parameter bestimmen zu können. Entsprechend ist der Einfluss der Lichtdynamik auf die Parameter der Wachstums-Licht-Beziehung noch weitgehend unbekannt. Dies beeinträchtigt auch die Zuverlässigkeit von Modellaussagen zur Phytoplanktondynamik unter Durchmischungsbedingungen. In dieser Dissertation sollen die experimentell gewonnenen Ergebnisse nicht auf ganze Ökosysteme extrapoliert werden; Ziel ist vielmehr ein verbessertes Verständnis der Prozesse, die die Lichtabhängigkeit des Phytoplanktonwachstums unter dynamischen Lichtbedingungen steuern.

Hierzu wurden im Tai-See und im Dreischluchten-Stausee (China) Experimente mit Phytoplanktongemeinschaften durchgeführt. Es wurden Proben entweder in konstanten Tiefen exponiert oder mit Liften vertikal zwischen Wasseroberfläche und verschiedenen Tiefen bewegt. Während das Lichtangebot in konstanten Tiefen nur dem Tagesgang der

Globalstrahlung folgte (hier als konstantes Licht bezeichnet), war das Phytoplankton in den bewegten Proben zusätzlich raschen Lichtfluktuationen ausgesetzt. Mit der Liftbewegung wurden mittlere Bedingungen in den Außenbahnen von Langmuir-Zellen simuliert, wobei eine Umlaufzeit von 20 Minuten gewählt wurde. Es wurden Wachstum, Photosynthese und (im Tai-See) Respiration gemessen. Zusätzlich wurde in Laborversuchen mit einem toxischen Stamm des Cyanobakteriums *Microcystis aeruginosa* (FACBH 1322) unter fluktuierendem und konstantem Licht Elektronentransportraten sowie Produktion und Verbrauch von Sauerstoff mit höherer zeitlicher Auflösung (1 min) gemessen.

Die Ergebnisse der vorliegenden Dissertation demonstrieren bedeutsame Effekte von Lichtfluktuationen auf die Ökophysiologie von Phytoplankton. Die Experimente unter halb-natürlichen Bedingungen im Tai-See und im Dreischluchten-Stausee zeigten ähnliche Muster. Die Wachstumseffizienz der Gemeinschaften nahm durch fluktuierendes Licht deutlich ab, überwiegend durch die veränderte Häufigkeitsverteilung der Lichtintensitäten, die zu verkürzten effektiven Taglängen führte. Zudem verringerten artspezifische Anpassungsmechanismen und lichtabhängige Verluste durch Respiration die Wachstumseffizienz bei fluktuierendem Licht. Die maximalen Wachstumsraten der Gemeinschaft unterschieden sich hingegen nicht zwischen den Ansätzen mit konstantem und fluktuierendem Licht. Bei Lichtsättigung des Wachstums unterschieden sich die Aufwendungen für die Biosynthese zwischen den beiden Lichtregimen. Unter konstantem Starklicht wurden die Photosynthese gehemmt und die Respiration zur Reparatur der Photosysteme erhöht. Fluktuierendes Licht hingegen vermied Lichthemmung, zwang die vertikal bewegten Algen aber zu alternativen Strategien der Lichtnutzung. Durch eine erhöhte Photosynthesekapazität konnten sie Starklicht nahe der Wasseroberfläche besser nutzen. *Microcystis aeruginosa* verbrauchte im Labor mehr Sauerstoff durch Respiration bei abnehmenden Lichtintensitäten kurz nach Starklicht. Innerhalb eines Lichtzyklus von 20 min stieg die Kompensationslichtintensität mit steigender Nettoproduktion bei Lichtsättigung. Diese Beobachtungen sind am besten durch eine Anreicherung von Photosyntheseprodukten bei ansteigender Lichtintensität und deren sofortige verstärkte Respiration für Erhaltungsumsatz und Biosynthese bei abnehmender Lichtintensität erklärbar. Im Tagesmittel führte eine verstärkte Photosynthese bei Lichtsättigung zu erhöhter Respiration bei Schwachlicht. Die Kompensationslichtintensitäten dominanter Arten im Tai-See stiegen mit deren artspezifischen maximalen Wachstumsraten. Durch diesen artspezifischen physiologischen Kompromiss unterschieden sich die dominanten Arten im See bezüglich ihrer

Lichtoptima. Unterschiedliche Strategien der Lichtnutzung (höhere maximale Wachstumsraten oder niedrigere Lichtansprüche) ermöglichten die Koexistenz verschiedener Arten entlang eines Gradienten der Intensität konstanten Lichts im Tai-See. Durch vertikale Durchmischung änderten sich die Strategien der Lichtnutzung innerhalb weniger Tage komplett. Die unterschiedlichen Anpassungsstrategien an fluktuierendes Licht vergrößerten die ökologischen Nischen der dominanten Arten und verhinderten ihre gegenseitige Verdrängung.

Insgesamt trägt diese Dissertation zum besseren Verständnis der Ökophysiologie von Phytoplankton unter Durchmischungsbedingungen bei. Dadurch werden verlässlichere Prognosen der Phytoplanktonentwicklung möglich, kurzzeitig in Kombination mit Wettervorhersagen und über lange Zeiträume durch Kopplung mit Klimaszenarien.

Schlagwörter: TaiHu, Dreischluchten-Stausee, funktionelle Eigenschaften, Zielkonflikte, fluktuierendes Licht, Lichtanpassung, Photosynthese, Respiration, Nischen-Aufteilung, Koexistenz unter wechselnden Bedingungen.

Being a researcher and breaking new ground is thrilling. It may be frustrating at times - because of the many difficulties one has to overcome in order to make this valuable contribution to the general knowledge and society.

As an idealist, I have always believed that being a researcher in ecology would contribute to making the world a better place for us, and also for our fellow plants and animals. At present, nothing is more questionable than our desire to make a step towards a future with more sustainable interactions between organisms and the environment. In this thesis, I wish to contribute my little piece to this dream that we all probably had in our childhood.

Our world is changing drastically (not to say dramatically) at a rapid pace. If Life, supported by phytoplankton can acclimate, why can't we?

À ma plus belle Madeleine

Preface

This publication-based PhD thesis presents the main outcomes of my research on the eco-physiological consequences of fluctuating light on phytoplankton. This work is based on two experiments performed in two Chinese freshwater systems (the Three Gorges Reservoir and Lake TaiHu) and one experiment performed in the laboratory. Two published papers, one under review and one in preparation are included in this thesis.

The first chapter is a comparison between the light-dependencies of photosynthesis and growth of phytoplankton incubated under defined mixing conditions and at fixed depths.
Köhler, J., Wang, L., Guislain, A., & Shatwell, T. (2018). Influence of vertical mixing on light-dependency of phytoplankton growth. *Limnology and Oceanography*, *63*(3), 1156-1167.

The second chapter investigates the poorly known interplay between photosynthesis, respiration and growth of phytoplankton communities under fluctuating light supply.
Guislain, A., & Köhler, J. (under review, Frontiers in Freshwater Science). Interplay between photosynthesis, respiration and growth of phytoplankton communities under vertical mixing.

The third chapter is an analysis of the interplay between photosynthesis and respiration under rapid light fluctuations in the laboratory.
Guislain, A., & Köhler, J. (in prep.) How does the cyanobacterium *Microcystis aeruginosa* respond to fluctuating light? A minute-based analysis of photosynthesis and respiration.

The fourth chapter describes how inter- and intra-specific variation of light acquisition traits under fluctuating light enhances non-equilibrium coexistence.
Guislain, A., Beisner, B. E., & Köhler, J. (2019). Variation in species light acquisition traits under fluctuating light regimes: implications for non-equilibrium coexistence. *Oikos, 128(5),* 716-728.

This work has been performed at the Leibniz-Institute for Freshwater Ecology and Inland Fisheries (Berlin) and is part of the Sino-German water supply project SIGN funded by the German Ministry of Education and Research (Grant number 02WCL1336D).

List of contents

List of abbreviations

Abbreviations	Description	Unit
PAR	Photosynthetically active radiation [400 - 700 nm]	See text
UV-A	Ultra-violet radiation A [320 - 400 nm]	-
UV-B	Ultra-violet radiation B [280 - 320 nm]	-
PSI and PSII	Photosystem I (or P700) and II (or P680)	-
ATP	Adenosine triphosphate	-
NADPH	Nicotinamide adenine dinucleotide phosphate	-
F0	Minimum fluorescence of dark-adapted phytoplankton	Relative unit
z_{eu}:z_{mix}	Euphotic zone to mixing depth ratio	Dimensionless
z_{crit}	Critical depth	m
TGR	Three Gorges Reservoir	-
SD / SE	Standard Deviation / Error	-
nlme	Nonlinear Mixed Effects Model	-
lme	Linear Mixed Effects Model	-
AIC	Akaike Information Criterion	-
Chl *a*	Chlorophyll *a*	$\mu g\ L^{-1}$
HPLC	High-Pressure Liquid Chromotography	-
PAM	Pulse Amplitude Modulation	-
F0, F20, F40, F80	Incubation bottles Fixed at 0, 20, 40 and 80cm	-
L50, L100, L180	Incubation bottles Lifted between 0 and 50, 100 and 180cm	-
μ	Growth rate	d^{-1}
μ_{max}	Maximal growth rate at saturating light	d^{-1}
α_{μ}	Growth efficiency at limiting light	$m^2\ E^{-1}$
α (only in Guislain et al. 2019)	Growth efficiency at limiting light	$m^2\ E^{-1}$
$PAR_{comp\mu}$	Compensation light intensity for growth (when $\mu = 0$)	$E\ m^{-2}\ d^{-1}$
I_{comp} (only in Köhler et al. 2018)	Compensation light intensity for growth (when $\mu = 0$)	$E\ m^{-2}\ d^{-1}$
PAR_{comp} (only in Guislain et al. 2019)	Compensation light intensity for growth (when $\mu = 0$)	$E\ m^{-2}\ d^{-1}$

Ik_{μ}	Onset of growth saturation	E m^{-2} d^{-1}
ETR	Electron transport rate	Relative unit
ETR_{max}	Maximal relative ETR at saturating light	Relative unit
α_{ETR}	Efficiency of ETR at limiting light	Relative unit or (μE $m^{-2}s^{-1}$)$^{-1}$
α_P (only in Köhler et al. 2018)	Efficiency of ETR at limiting light	Relative unit or (μE $m^{-2}s^{-1}$)$^{-1}$
Ik_{ETR}	Onset of ETR saturation	μE m^{-2} s^{-1}
Ik_P (only in Köhler et al. 2018)	Onset of ETR saturation	μE m^{-2} s^{-1}
P_{max}	Maximal daily net oxygen production rate at saturating light	μg O_2 d^{-1} μg^{-1} Chl *a*
α_P	Efficiency of daily net oxygen production at limiting light	μg O_2 d^{-1} μg^{-1} Chl *a* E^{-1} m^2
PAR_{compP}	Compensation light intensity for daily net oxygen production	E m^{-2} d^{-1}
Ik_P	Onset of light saturation for daily net oxygen production	E m^{-2} d^{-1}
$P_{max,20}$	Maximal oxygen net oxygen production rate at saturating light (20 min timescale)	ng O_2 μg^{-1} Chl *a* s^{-1}
$\alpha_{P,20}$	Efficiency of oxygen net production at limiting light (20 min timescale)	ng O_2 μg^{-1} Chl *a* μE^{-1} m^{-2}
$PAR_{compP,20}$	Compensation light intensity for net oxygen production(20 min timescale)	μE m^{-2} s^{-1}
$Ik_{P,20}$	Onset of light saturation for net oxygen production (20 min timescale)	μE m^{-2} s^{-1}
P_{max} (in Guislain and Köhler, in prep.)	Maximal net oxygen production rate at saturating light (minute-based)	ng O_2 min^{-1} μg^{-1} Chl *a*
α_P (in Guislain and Köhler, in prep.)	Efficiency of net oxygen production rate at limiting light (minute-based)	Relative unit
PAR_{compP} (in Guislain and Köhler, in prep.)	Compensation light intensity for net oxygen production rate (minute-based)	μE m^{-2} s^{-1}

General introduction

As the major primary producers on Earth, phytoplankton are responsible for about half of the global net production of photosynthetic organisms (Field et al. 1998). Thus, they provide a major carbon source to aquatic food webs, strongly influence biogeochemical cycles and may impair the usability of surface waters (Falkowski et al. 1998, Falkowski 2012, Litchman et al. 2015). The growth rate of a given phytoplankton species depends mainly on temperature and supply of nutrients and light. The light supply also influences both the temperature (Edwards et al. 2016) and the nutrient dependency of growth (Litchman et al. 2004).

Light is an electromagnetic radiation delivered in discrete energetic packages called photons. The energy of a photon is inversely related to its wavelength:

$$\varepsilon = \frac{\mathrm{h\,c}}{\lambda}$$

where ε is the photon or quantum energy, h is the Planck's constant, c is the constant of light velocity and λ is the wavelength of the light waves (Kirk 1994).

Proportionally to their energy, photons increase the energy states of electrons in the absorbing molecule or chromophore. The latter is present in the pigments of prokaryotic (cyanobacteria) and eukaryotic algae that absorb light at specific wavelengths (Fig. 1). Only the light spectrum comprised between 400 and 700 nm can be used for photosynthesis and is hence called Photosynthetically Active Radiation or PAR.

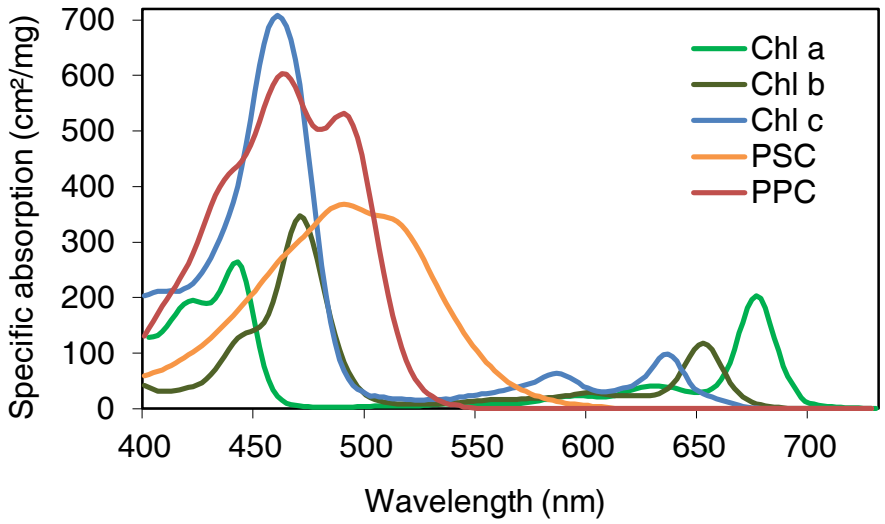


Figure 1. "Unpacked" specific absorption coefficients of major phytoplankton pigment groups (after Bidigare et al. 1990). Chl: Chlorophyll; PPC: photoprotective carotenoids; PSC: photosynthetic carotenoids.

1. From light absorption to phytoplankton growth – A brief overview

Absorbed PAR leads to a raise in the excitation status of algal pigments. After excitation, pigments need to reach their more stable ground state of energy. Hence, three de-excitation pathways may occur: light energy can be used to drive photosynthesis (photochemistry), it can be dissipated as heat (non-photochemical quenching) or re-emitted as light *i.e.* chlorophyll fluorescence (Müller et al. 2001, Falkowski and Raven 2007, Lin et al. 2016). Under optimal laboratory growth conditions, about 65% of absorbed photons by marine phytoplankton are used for photochemistry, less than 35% are dissipated as heat and the rest is re-emitted as fluorescence. *In situ*, the proportions are reversed, with 35% of absorbed photons used for photochemistry, 60% dissipated as heat and the rest re-emitted as fluorescence (Lin et al. 2016).

Primary production in aquatic ecosystems depends on photosynthesis by phytoplankton which assimilates inorganic carbon into biomass. The capture of light energy for photosynthesis is achievable via the photosynthetic pigments pool (also called antennae) located within the photosystems (PSII and PSI, named after their order of discovery). The

excited electrons are then transmitted to the reaction centers of the photosystems to transfer these energized electrons through the electron transport chain (Fig. 2, Appendix-Fig.1).

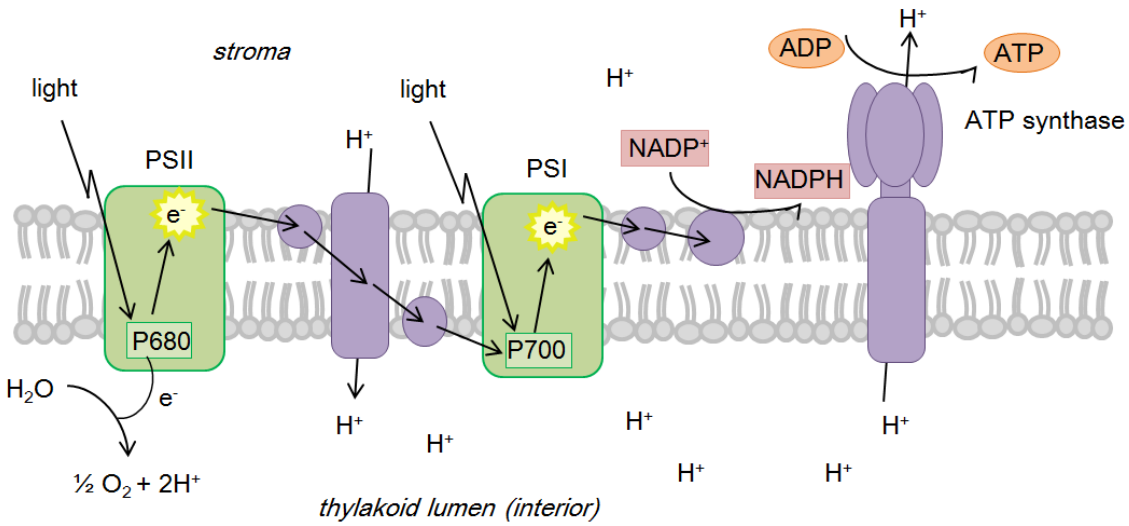


Figure 2. Photosynthetic electron transport chain (Falkowski and Raven 2007).

Produced Nicotinamide Adenine Dinucleotide Phosphate (NADPH) and Adenosine TriPhosphate (ATP) are then used to fix carbon as carbohydrates within the light-independent Calvin-Benson cycle (Appendix-Fig.2). Overall, photosynthesis can be simply described as:

$$6CO_2 + 6H_2O \xrightarrow{\text{PAR + nutrients}} C_6H_{12}O_6\,(= \text{carbohydrate}) + 6O_2$$

Dark respiration is the opposite reaction to photosynthesis and produces back ATP and NADPH from carbohydrates. Dark respiration is necessary for phytoplankton growth and is a significant link between photosynthesis and phytoplankton growth. It provides carbon skeletons required for biosynthesis (Raven and Beardall 2003). As suggested by its name, it was believed that dark respiration was active only in the dark, but few studies indicate higher rates of respiration in the light compared to the dark (Grande et al. 1989, Luz et al. 2002). Although not directly involved in biosynthesis, it worth mentioning that dark respiration is not the only respiratory pathway responsible for the reduction of oxygen. There is also the Mehler reaction (or chlororespiration) and the photorespiration (Appendix-Fig.3).

2. Nonlinear light-dependency of phytoplankton growth

Phytoplankton growth is nonlinearly related to light and could usually be partitioned in three: a proportional increase at limiting light supply, followed by a transition region around the onset of saturation, to finally reach a plateau at saturating light intensities. From such growth-light relationships, one may extract demographic traits of a population (or "parameters" for a community - see Violle et al. 2007) that can be seen to represent light acquisition traits as they provide reliable indicators of the ability of one species to grow at certain light intensities (Litchman et al. 2012). Traits include: the initial slope of the growth-light curve α_μ which reflects the growth efficiency at limiting light, the maximum growth rate at saturating light μ_{max} and $PAR_{comp\mu}$ the light intensity at zero growth, the so-called compensation light intensity (Fig. 3). The onset of growth saturation Ik_μ is then calculated as: $Ik_\mu = \mu_{max} / \alpha_\mu + PAR_{comp\mu}$.

These light acquisition traits integrate many underlying physiological processes that are sensitive to light levels. μ_{max} and α_μ are mainly driven by the energy allocated to growth (*e.g.* ribosomes) and light-harvesting machinery (*e.g.* chlorophyll complexes (chlorophyll : C ratio), accessory pigments, effective absorption cross-section) respectively (Langdon 1988, Klausmeier et al. 2004, Litchman 2007, Talmy et al. 2013). $PAR_{comp\mu}$ is driven by the balance between photosynthesis at limiting light (and thus, light-harvesting machinery) and maintenance respiration (Langdon 1988, Box 1). $PAR_{comp\mu}$ is primarily affected by maintenance respiratory costs (Langdon 1988).

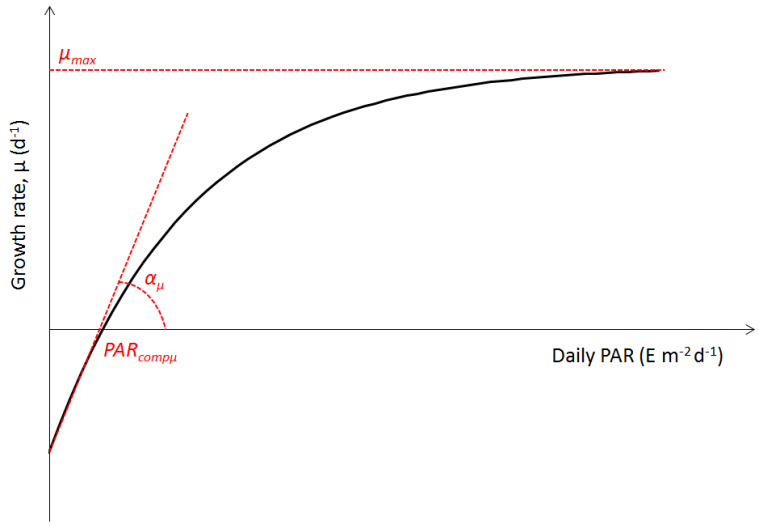


Figure 3. Graphical description of the growth-light traits of a population.

Hence, light acquisition traits offer a promising mechanistic link between the environment and community dynamics in both marine (Edwards et al. 2013a) and freshwater (Edwards et al. 2013b) ecosystems. However, the light-dependency of growth has been mostly measured for phytoplankton species exposed to constant PAR supply under laboratory conditions. Until now, it remains largely unknown how light fluctuations influence μ_{max}, α_{μ} and $PAR_{comp\mu}$.

Box 1

In growth energetics, it is assumed that respiratory costs are attributed to 1/ maintenance metabolic costs, independent of growth and 2/ costs of biomass (or cell) synthesis, dependent on growth (Geider and Osborne 1989). Maintenance metabolism (at growth μ=0) includes for instance turnover of macromolecules and motility; whereas the cost of biosynthesis is related to synthesis of cell structural and functioning components. However, it seems that some processes included into maintenance costs (*e.g.* protein turnover) respond to changing environment (Pirt 1975). As described in details by Geider and Osborne (1989), variations in maintenance respiratory costs may also be considered as changes in the efficiency of biosynthesis.

3. Light fluctuations in nature and effects on phytoplankton growth

In the water column, irradiance exponentially declines with increasing optical depth, which is the product of depth and vertical light attenuation. The light intensity in the water column is expressed following the Lambert-Beer's law:

$$I_z = I_0 * e^{-kz}$$

where I_z is the light intensity at depth z (m), I_0 is PAR at the water surface and k the light attenuation coefficient (m^{-1}). The light attenuation depends on the optical properties of pure water and absorption of particles and dissolved colored materials (Kirk 1994).

Phytoplankton (coined by the German physiologist Viktor Hensen from ancient Greek *planktós i.e.* "wandering") are incapable to overcome water movements. Surface layers or

even whole water bodies are frequently mixed by many physical processes acting at various timescales (Table 1). Thus, phytoplankton experience light of fluctuating intensity according to the exponential decline of light intensity with depth (Kirk 1994). In lakes and larger water bodies, moderate wind intensities suffice to generate Langmuir cells, named after the author who first described them (Langmuir 1938). These circular counter-rotating eddies often dominate other turbulent processes, especially in large water bodies (Harris and Piccinin 1977, Thorpe 2004). Light fluctuations applied in this PhD thesis correspond to a typical 20 min full overturn of Langmuir cells (Denman and Gargett 1983, Schubert and Forster 1997, Riddle and Lewis 2000, Thorpe 2004). Within a Langmuir cell, phytoplankton may experience light saturation (or light inhibition) at the air-water interface and darkness if the mixing depth is greater than the euphotic zone[1].

Table 1. Main light fluctuations in water bodies (adapted from Ferris and Christian 1991). The approximate time required for completing a cycle of movement is specified.

Cause	Approximate timescale
Seasons	year
Storm associated phenomena	1-14 days
Diurnal pattern	24 hours
Cloud	30 seconds - 4 minutes
Floating macrophytes, edge shadows	sec - min
Surface waves (flicker effect)	seconds (0.1-10)
Tides	12.4 hours - 13.7 days
Internal waves (<30m displacement)	3 min-16.7 hours
Langmuir circulation (<10m displacement)	20-30 min
Turbulent mixing (10m displacement)	30 min - 11.6 days

Light fluctuations may influence phytoplankton growth because of the nonlinearity of the photosynthesis and growth-light relationships, but also because of photoacclimation to dynamic light. Effects of nonlinearity and photoacclimation are briefly introduced in the following paragraphs.

[1]The euphotic zone has been traditionally assumed to be the depth where irradiance equals 1% of the surface photosynthetically active radiation - the depth below which no photosynthesis may occur (Ryther 1956, Marra et al. 2013).

3.1. Photoacclimation processes

Phytoplankton in mixing water columns may be imperfectly adapted to the instantaneous light conditions and may photoacclimate to the changes in PAR. Photoacclimation is defined as the reversible phenotypic adjustments in response to variations of light (Falkowski and LaRoche 1991). Extensive knowledge in the field and laboratory has been built regarding the dynamic responses of phytoplankton to increased or decreased light intensities (Ferris and Christian 1991 for review). Yet, the ability of algae and cyanobacteria to cope with fluctuating light is not always well known. Some acclimation processes received much attention such as: non-photochemical quenching through xanthophyll cycle (*e.g.* Lavaud et al. 2007, Brunet and Lavaud 2010), state transitions (*e.g.* Mikko et al. 2006), light absorption (*e.g.* Stramski et al. 1993, Nicklisch 1998) or photosynthesis (*e.g.* Marra 1978, Walsh and Legendre 1983, Fietz and Nicklisch 2002). On the contrary, much less attention was given to respiration of phytoplankton under fluctuating light (Richardson 1983, Beardall et al. 1994, Avendaño-Coletta and Schubert 2005). This lack of knowledge is especially true for the interplay between photosynthesis and respiration. Phytoplankton are a very diverse (*c.a.* 3000-5000 of marine species in the ocean (Sournia et al. 1991, Reynolds 2006)) polyphyletic group coming from both prokaryotic (cyanobacteria) and eukaryotic domains. Species differ in many aspects of their cellular components, physiology and evolutionary history (Glover et al. 1987, Gregory 2001, Yoon et al. 2004) and this diversity is also expressed through the photoacclimation processes (*e.g.* Litchman 2000, Wagner et al. 2006, Dimier et al. 2009, Shatwell et al. 2012). Photoacclimation to fluctuating light is species (potentially even clonal-dependent, Kardinaal et al. 2007), but also timescale dependent (Litchman 2000, MacIntyre et al. 2000). Hence, phytoplankton may develop under varying light levels only if their capacity to adjust their physiology is not outpaced by PAR changes (MacIntyre 2000). For these reasons it is very challenging to adequately predict the effects of fluctuating light intensities on phytoplankton development.

Overall, it seems reasonable to think that acclimation to fluctuating light is driven in part by a tradeoff between resource allocation to mechanisms that protect against high light and growth efficiency at low light intensities (MacIntyre et al. 2002, Talmy et al. 2013). Phytoplankton in a turbulent surface layer is potentially forced to avoid light inhibition of its photosystems near the water surface. For this, vertically mixed phytoplankton have been shown to preferably enhance their photosynthesis to benefit from rapid surface light peaks

(Kana and Glibert 1987) while investing a significant amount of energy into protection against light inhibition (Dubinsky and Stambler 2009, Talmy et al. 2013). However, mechanisms that protect against high light diminish the efficiency of photosynthesis and growth at low light intensities (MacIntyre et al. 2002). In general, it is thought that fluctuating light may increase physiological losses like respiration (Beardall et al. 1994) or exudation (Cosper 1982). On the contrary, algae receiving relatively constant low light intensities would generally allocate more energy into an efficient light harvesting machinery (*e.g.* high chlorophyll : C ratio) but may be growth saturated at lower light intensities.

3.2. Growth-light nonlinearity

Photosynthesis and growth are nonlinearly related to light and thus depend on the mean light intensity and also on the temporal light distribution (Litchman 2000). Saturating light intensities near the water surface allow for less carbon fixation per available photon than under sub-saturating light. Therefore, growth should be less efficient when the light supply fluctuates between very low and saturating or even inhibiting intensities than when the light supply is constant and sub-saturating at the same mean intensity. Moreover, under nutrient-replete steady-state conditions, phytoplankton grow until self-shading reduces the mean light intensity in the mixed layer to $PAR_{comp\mu}$. Thus, if algae are transported below the depth at which the irradiance equals $PAR_{comp\mu}$, the effective daylength is shortened and may reduce growth rates in a species-specific fashion (Nicklisch 1998, Nicklish and Fietz 2001, Shatwell et al. 2012). The effects of nonlinearity and reduction of photoperiod can be estimated by photosynthesis models of sufficient temporal resolution (*e.g.* Cianelli et al. 2004, Ross et al. 2011) if the vertical movement of the algal cells is known.

The variation of the light-dependency of phytoplankton growth under fluctuating light has strong ecological consequences. The effects of fluctuating light on the species non-equilibrium coexistence are addressed in this thesis.

4. Effects of fluctuating light on the non-equilibrium coexistence

Spatial and temporal heterogeneity offer niche opportunities for species with different ecological strategies to develop and potentially coexist (Chesson and Case 1986, Chesson 2000). Spatial heterogeneity reduces niche overlap, enabling coexistence by favouring different species in different local environments through environmental filtering. For instance, in a stratified eutrophic lake, phytoplankton must cope mostly with the exponential decline of irradiance with depth. Temporal heterogeneity can also promote species coexistence through differential nonlinear species-specific responses to a fluctuating limiting factor. Different species may thus dominate the community at times when they are able to most actively use the resource (Chesson 2000, Adler et al. 2013).

In aquatic ecology, the coexistence of several phytoplankton species in a seemingly homogeneous environment was originally characterized as the “Paradox of the Plankton” (Hutchinson 1961). Very few studies have focused on the effects of fluctuating light on species competition and coexistence (Litchman 1998, Flöder et al. 2002), solely investigating species diversity and/or species-specific growth rates at either low or high light levels. Light acquisition traits calculated from traditional growth-constant light relationships measured in the laboratory have been used to explain phytoplankton distribution along environmental light gradients (Schwaderer et al. 2011). However, the variation of species light acquisition traits under fluctuating light should alter interspecific competition, promote coexistence or exclude inefficient species in diverse phytoplankton communities. In general, it is still unknown how species light acquisition trait variation under fluctuating light may alter niche partitioning and thus species coexistence in bulk phytoplankton communities.

5. Approach and questions

The light-dependency of growth has been measured for many phytoplankton species at constant irradiances in the laboratory. As explained previously, this relation is characterized by a small number of basic parameters: the compensation light intensity $PAR_{comp\mu}$, where production and losses are balanced, the growth efficiency at sub-saturating light α_{μ}, and the

maximum growth rate at saturating light μ_{max}. An additional parameter may describe growth inhibition at inhibiting light intensities. These light acquisition traits calculated from traditional growth-constant light relationships have been used to explain phytoplankton distribution along environmental light gradients (Schwaderer et al. 2011). Predictions of phytoplankton distribution and aquatic ecosystem models are as accurate as the growth-light parameters used. Very few studies (Nicklisch et al. 2008, Shatwell et al. 2012) measured growth under fluctuating light at a sufficient number of mean light intensities to estimate the parameters of the growth-light relationship. Therefore, the influence of light dynamics and factors of variations on μ_{max}, α_{μ} and $PAR_{comp\mu}$ are still largely unknown. By extension, accurate predictions of phytoplankton development under fluctuating light exposure remain difficult to make. Nicklisch et al. (2008) and Shatwell et al. (2012) performed laboratory experiments on monocultures and allowed a period of acclimation (7- 14 days depending on the mean irradiance tested) to a certain mean irradiance before measurements. Although providing interesting outcomes, this experimental approach remains far from reality and may dampen phytoplankton response to fluctuating light mostly because: 1/ Species in communities generally diverge more in resource use to reduce niche overlap than in a monoculture setup (Lawrence et al. 2012) 2/ Measuring growth in the laboratory after a period of acclimation to a certain PAR lowers the importance of rapid photoacclimation to the much more unpredictable natural light conditions.

To get closer to natural conditions, we used an incubation method first introduced by Nixdorf and Behrendt (1991). We mimicked vertical mixing and induced fluctuating light regimes by computer-controlled motion of subsamples from a lake phytoplankton community (under nutrient-replete conditions and drastically reduced grazing pressure). In this thesis I focus on the common, relatively regular Langmuir cells which need, depending on wind speed and mixing depth, a few minutes to 1 h per revolution (Denman and Gargett 1983, Schubert and Forster 1997, Thorpe 2004). We used this experimental approach in the manuscripts presented in Chapters 1, 2 and 4.

The nonlinear light-dependency of growth may differ between constant and fluctuating light because of the different frequency distribution of light and/or acclimation processes. First, I intend in this thesis to assess the influence of light dynamics on μ_{max}, α_{μ} and $PAR_{comp\mu}$ of communities and dominant species. My first research question is the following:

Q1. How does the light-dependency of growth of phytoplankton communities and dominant

species incubated under defined mixing conditions differ from the one at fixed depths?
I hypothesize:
H1. Growth-light parameters (for community) and traits (for population) are different between the traditional constant light incubation and under fluctuating light.

Variation in photosynthesis is one of the most studied responses of phytoplankton to fluctuating light - and this thesis is not an exception. Nonetheless, both photosynthesis and dark respiration are essential for biosynthesis and are dynamic responses of mixed algae to mean and temporal distribution of irradiance at vertical mixing (Ferris and Christian 1991). These physiological processes are tightly connected, but their interplay is not well understood. As a matter of fact, previous investigations on the effects of fluctuating light mostly focused on either growth, photosynthesis or physiological losses. We try to fill this gap and we address the following question:
Q2. What is the interplay between the light-dependent photosynthesis, respiration and growth of phytoplankton communities under fluctuating light exposure?
From this question emerges another one that can hardly be answered by an experiment performed under semi-natural conditions:
Q3. What is the interplay between photosynthesis and respiration at rapid light fluctuations, *i.e.* at the timescale of a Langmuir cell?
Several authors argued that mixed phytoplankton take advantage of cyclic periods in effective darkness for rapidly triggering respiration, relaxing their photosystems and efficiently coupling light and dark reactions during the day. But, dark respiration may also be enhanced in the light. To analyze the interplay between photosynthesis and respiration under rapid light fluctuations, we performed a laboratory experiment with a monoculture of *Microcystis aeruginosa* grown under 20 min period light fluctuations (Chapter 3).
I hypothesize:
H2. The interplay between photosynthesis, respiration and growth is different between constant and fluctuating light exposures. Further, at the timescale of a Langmuir cell, phytoplankton enhance their maximum photosynthesis at high irradiance and rapidly increase dark respiration for maintenance and biosynthesis.

Finally, in this thesis I address the ecological consequences of fluctuating light exposure on phytoplankton. The growth-light traits are plastic and may have different values between

incubations under constant and fluctuating light. This should affect species coexistence. Hence:

Q4. How does species light acquisition trait variation under fluctuating light alter niche partitioning and thus species coexistence in bulk phytoplankton communities?

I hypothesize:

H3. The variation of species-specific light traits under fluctuating light enhances non-equilibrium coexistence in turbulent systems**.**

Declaration of contributions

Chapter 1

Published as: *Influence of vertical mixing on light-dependency of phytoplankton growth.*

Authors: Köhler, J., Wang, L., Guislain, A., & Shatwell, T.

Published in *Limnology and Oceanography* (2018), *63*(3), 1156-1167.

JK lead writer, designed and performed the experiment, analysed the data and interpreted the results. LW participated in the sampling and measurements in the lake. AG contributed to the manuscript. TS analysed the data, and contributed to the manuscript.

Chapter 2

Submitted as: *Interplay between photosynthesis, respiration and growth of phytoplankton communities under vertical mixing.*

Authors: Guislain, A., & Köhler, J.

Under review in *Frontiers in Freshwater Science*.

AG lead writer, designed and performed most of the experiment under the guidance of JK. AG analysed the data and interpreted the results. JK designed the experiment, participated in the sampling in the lake, performed the fluorometric measurements and commented on the manuscript.

Chapter 3

In preparation as: *How does the cyanobacterium Microcystis aeruginosa respond to fluctuating light? A minute-based analysis of photosynthesis and respiration.*

Authors: Guislain, A., & Köhler, J.

AG lead writer, designed and performed the experiment, analysed the data and interpreted the results. JK designed the experiment and commented on the manuscript.

Chapter 4

Published as: *Variation in species light acquisition traits under fluctuating light regimes: implications for non-equilibrium coexistence.*

Authors: Guislain, A., Beisner, B. E., & Köhler, J.

Published in *Oikos* (2019)*, 128(5),* 716-728.

AG lead writer, designed and performed most of the experiment under the guidance of JK. AG counted the phytoplankton, analysed the data and interpreted the results. JK designed the experiment, participated in the sampling in the lake and performed the fluorometric measurements. JK and BEB commented on the manuscript.

Berlin, 18th November 2019

Alexis Guislain: _____ Dr. Jan Köhler: _____

Chapter 1

Manuscript title:

Influence of vertical mixing on light-dependency of phytoplankton growth.

Jan Köhler[1], Lan Wang[2], Alexis Guislain[1,3], Tom Shatwell[1]

[1]Leibniz-Institute for Freshwater Ecology and Inland Fisheries, Department of Ecosystem Research, Berlin, Germany.

[2]Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China.

[3]University of Potsdam, Department of Ecology and Ecosystem Modelling, Potsdam, Germany.

Published as:

Köhler, J., Wang, L., Guislain, A., and Shatwell, T. (2018). Influence of vertical mixing on light-dependency of phytoplankton growth. *Limnol. Oceanogr.* 63, 1156–1167. doi:10.1002/lno.10761.

Abstract

Phytoplankton growth depends not only on mean intensity but also on the dynamics of the light supply. In surface mixed layers, phytoplankton may rapidly move between strong light and almost darkness. The nonlinear light-dependency of growth may differ between constant and fluctuating light because of the different frequency distribution of light and/or acclimation processes. The present study compares for the first time light-dependency of photosynthesis and growth of phytoplankton communities *in situ* under defined mixing conditions and at fixed depths. Maximum growth rates per day were not significantly different, but the growth efficiency was much higher under constant light than under fluctuating light of sub-saturating daily irradiance. Phytoplankton incubated under fluctuating light needed about 3 times higher mean daily irradiances to balance photosynthesis and losses than under constant light. The difference in growth efficiency was mostly caused by the different frequency distribution of underwater light, as was estimated by a photosynthesis model of sufficient temporal resolution. The present study indicates a considerable overestimation of phytoplankton growth at sub-saturating light in well-mixed water layers by the common growth measurements under constant light. This implies an underestimation of the compensation light intensities and respective overestimations of the critical mixing depths.

Keywords: Algal dynamics, Turbulent mixing, Functional traits, Photosynthesis, Three Gorges reservoir

Introduction

Planktonic algae contribute about 46% to global biogenic carbon fixation and thus play a crucial role for the global CO_2 budget (Field et al. 1998). They provide a major carbon source to aquatic food webs, strongly influence the functioning of aquatic ecosystems and may impair the usability of surface waters. The growth rate of a given algae species depends mainly on temperature and supply of nutrients and photosynthetically available radiation (PAR). The PAR supply influences both the temperature dependency (Edwards et al. 2016) and the nutrient dependency of growth (Litchman et al. 2004). Compared to nutrients, light is a more dynamic resource. Seasonal and diurnal changes as well as cloud cover influence the irradiance at the water surface. In the water column, irradiance exponentially declines with increasing optical depth, which is the product of depth and vertical light attenuation. Surface layers or even whole water bodies are frequently mixed by wind stress or heat loss. Even moderate wind intensities suffice to generate circular, counter-rotating eddies (Langmuir cells), which are the rule rather than the exception in larger water bodies (Harris & Piccinin 1977).

Suspended algae experience light of fluctuating intensity during transport in the mixing layer (Kirk 1994). Photosynthesis and growth are nonlinearly related to light. Therefore, they depend not only on the mean intensity but also on the frequency distribution of received light intensities (Litchman 2000). Phytoplankton spend parts of the day in darkness if the mixing depth exceeds the depth of the euphotic zone. The shortened effective daylength causes respective declines in growth rates (Shatwell et al. 2012). Saturating light intensities near the water surface allow for less carbon fixation per available photon than under sub-saturating light. Therefore, growth should be less efficient when the light supply fluctuates between very low and saturating or even inhibiting intensities than when the light supply is constant and sub-saturating at the same mean intensity. This effect of nonlinearity can be estimated by photosynthesis models of sufficient temporal resolution (*e.g.* Cianelli et al. 2004, Ross et al. 2011) if the vertical movement of the algal cells is known. The second type of factor influencing growth efficiency under turbulent mixing is more difficult to assess: Phytoplankton in mixing water columns may be imperfectly adapted to the instantaneous light conditions if changes in PAR outpace their capacity to acclimate. Phytoplankton in a turbulent surface layer is potentially forced to avoid light inhibition of its photosystems near the water surface. However, mechanisms that protect against strong light diminish the

efficiency of photosynthesis and growth at low light (MacIntyre et al. 2002). Fluctuating light may increase physiological losses like respiration (Beardall et al. 1994) or exudation (Cosper 1982). Light flashes (Phillips & Myers 1954, Abu-Gosh et al. 2015) and periodical relaxing from otherwise inhibiting irradiance (Ibelings et al. 1994, Neale et al. 1998, Helbling et al. 2013) may also favour phytoplankton growth. Phytoplankton species adapted to moderate but dynamic irradiance ("mixers" *sensu* Cullen & MacIntyre 1998) may increase their photosynthesis when rapidly exposed to high irradiance (Kana & Glibert 1987). The ability to acclimate to fluctuating light is species-specific (*e.g.* Ibelings et al. 1994, Litchman 2000, Shatwell et al. 2012) and not always well-known. So far, we cannot adequately predict the effects of changed mixing conditions on phytoplankton development.

The light-dependency of growth has been measured for many phytoplankton species at constant irradiances (*e.g.* Jitts et al. 1964, Schwaderer et al. 2011). This relation is characterized by a small number of basic parameters: the compensation light intensity I_{comp}, where production and losses are balanced, the growth efficiency at sub-saturation light α_{μ}, and the maximum growth rate under saturating light μ_{max}. An additional parameter may describe growth inhibition at strong light. Very few studies (Nicklisch et al. 2008, Shatwell et al. 2012) measured growth under fluctuating light at a sufficient number of mean light intensities to estimate the parameters of the growth-irradiance relationship. Therefore, the influence of light dynamics on μ_{max}, α_{μ} and I_{comp} is still largely unknown.

Each of the different response mechanisms matches only a limited range of light frequencies (e.g., Cullen & Lewis 1988). This study focuses on the common, relatively regular Langmuir cells which need, depending on wind speed and mixing depth, a few minutes to one hour per revolution (see Denman & Gargett 1983, Schubert & Forster 1997, Thorpe 2004).

We tested the following hypotheses for such mixing conditions:

H1. Differences in growth efficiency of phytoplankton between stagnant and turbulent conditions are mostly explainable by the different frequency distribution of the received light.

H2. At the same daily PAR, growth rates of phytoplankton are similar in mixed and in stratified water columns only at similar frequency distributions of light, *i.e.* at low optical depths. This would suggest similar maximum growth rates at mostly saturating irradiances.

H3. At deeper mixing, shortened effective daylength, the higher percentage of saturating or even inhibiting intensities and additional energy required to adapt to light fluctuations cause slower growth than under constant light of the same mean intensity. As a result, daily light requirements for zero growth (I_{comp}) and for light-saturated growth (Ik_{μ}) should be higher under fluctuating light than under constant light.

To test these hypotheses, we performed two series of experiments at the Xiangxi bay of the Three Gorges reservoir, China. We compared growth rates and photosynthesis of phytoplankton samples which were either vertically moved or incubated at fixed depths of similar daily irradiance. This "yo-yo technique" (Köhler 1997, Köhler et al. 2001, Mitrovic et al. 2003) combines the well-defined mixing conditions and avoided settling losses of laboratory experiments and the natural light field of mesocosms.

Methods

Site description

The experiments were performed in the Xiangxi Bay of the Three Gorges Reservoir, China, about 38 km upstream of the dam. A float anchored about 140 m offshore (31°06`50``N 110°46`52``E) was used for experimental installations, measurement of vertical profiles and a monitoring station (Wang et al. 2011a). The whole reservoir has a surface area of 1,080 km² and a length of about 600 km at normal water level (175 m a.s.l.). In Xiangxi Bay, high nutrient concentrations and sufficiently long residence time of water enable severe phytoplankton blooms in spring and summer (Wang et al. 2011b, Liu et al. 2012).

Experimental approach

Experiments started at sunrise of April 4 and 10, 2011 and lasted for 96 h each. Water was sampled from 0.3 m depth and pre-filtered (64 µm) to remove large zooplankton. In each experiment, 18 bottles (Duran glass, 280 mL) were filled from the same bucket. They were incubated in triplicate either at a fixed depth or vertically moved by a computer-controlled lift. The stationary samples were fixed at depths of about the same daily irradiances as received by their moved counterparts. The lift simulated a circular path from the water surface to 3, 7 or 14 m depth (10 m during the second experiment) with a 20 minute period. The applied sinusoidal variation of vertical velocity is an approximation to more complex turbulent processes which may cause accumulation of buoyant algae in near-surface

windrows (Denman & Gargett 1983), stronger downward than upward velocities (Gargett & Wells 2007) or extended residence time in the middle of the Langmuir cell (Thorpe 2004). The revolution period was chosen according to Denman & Gargett (1983), Schubert & Forster (1997) and Riddle & Lewis (2000), who found periods of about 20 minutes for full overturn in typical Langmuir cells. Subsamples of 50 mL were taken from each bottle after thorough homogenisation at sunrise of days 2-4. Bottles were topped up with filtered reservoir water (Whatman GF/C) to avoid nutrient limitation and self-shading and were re-incubated within 20 minutes.

Phytoplankton biomass and species composition

Samples were transferred in a dark cooler to the nearby laboratory. After at least 20 minutes dark adaptation, three subsamples were taken from each bottle to measure chlorophyll fluorescence yields at very low light intensity (F_0) in a Phyto-PAM fluorometer (Walz, Germany). F_0 values were converted into chlorophyll *a* (chl *a*) concentrations using HPLC-based calibration factors. Additionally, subsamples were fixed with Lugol´s solution. The abundance of dominant phytoplankton taxa was calculated after counting 300-800 cells per sample under an inverted microscope (Utermöhl 1958). Relevant dimensions of at least 20 cells per species were measured to calculate biovolumes. Total phytoplankton biovolume was closely correlated to PAM-derived chl *a* (r^2=0.93, n=14, p<0.001). The specific chl *a* content (chl *a* / biovolume) was not significantly different between vertically moved and stationary samples (p=0.30). The phytoplankton in the first experiment was initially dominated by dinoflagellates (*Peridinopsis niei*) and, to a much lesser extent, by green algae (*Pandorina morum*, *Eudorina elegans*), whereas each diatom taxon (*Asterionella formosa*, *Synedra* spec., *Fragilaria* spec., centric diatoms) contributed less than 1% to the total biovolume. Phytoplankton in the second experiment mainly consisted of *Fragilaria* spec. and *Synedra* spec. (74%), *Peridinopsis niei* and centric diatoms.

Photosynthesis

Rapid photosynthesis-light curves were measured in the Phyto-PAM immediately after F_0. Relative electron transport rates (ETR) were quantified at 11 PAR intensities (1-600 µE m^{-2} s^{-1}) after 30 seconds adaptation at each intensity. Efficiency of light-limited ETR (α_P), maximum relative electron transport rates (ETR_{max}) and the transition parameter from limiting to saturating light (I_{KP} = ETR_{max}/ α_P) were fitted using the model of Webb et al. (1974). This

model, α_P, ETR_{max} and the diurnal courses of PAR received by the vertically moved or the stationary algae were used to calculate relative electron transport rates of each sample every 75 s which were afterwards integrated per day. The time step of 75 seconds corresponds to the velocity segments of the circular path simulated by the lifts.

Abiotic conditions

Vertical profiles of temperature, chlorophyll fluorescence, oxygen concentration and photosynthetically active radiation were measured at 0.5 m intervals from the water surface to 20 m depth at 10 am and 4 pm each day using a YSI 6600 EDS multiprobe (Yellowsprings) and a Li-192 SA (LiCor) quantum sensor, respectively. The mean coefficient of vertical light attenuation (ε) was calculated by applying the Lambert-Beer law. A moored monitoring station recorded downwelling PAR above the water surface with a cosine-corrected quantum sensor (Li-190), as well as air temperature, wind speed and humidity (meteoMS, ecotech, Germany).

Calculations and statistics

Growth rates (d^{-1}) were calculated from changes in chl *a* taking into account dilution after sampling of the previous day:

$$\mu_i = \ln\left[\frac{\text{Chl } a_{i+1}}{\text{Chl } a_i * \frac{(V-50)}{V}}\right] \quad \text{eq. 1}$$

where chl a_i is the chlorophyll *a* concentration at day i and V the volume of the bottle in mL. The light-dependency of growth was modelled according to Webb et al. (1974) as

$$\mu = \mu_{max}\left[1 - \exp\left(\frac{-\alpha_\mu(Iz - I_{comp})}{\mu_{max}}\right)\right] \quad \text{eq. 2}$$

where μ_{max} is the growth rate under saturating light (d^{-1}), α_μ the growth-efficiency under sub-saturating light ($m^2\ E^{-1}$), I_z is the intensity of PAR at depth z and I_{comp} the compensation light intensity at zero growth ($E\ m^{-2}\ d^{-1}$). The model can also be formulated in terms of $I_{k\mu} = \mu_{max} / \alpha_\mu + I_{comp}$. Model parameters were estimated using nonlinear least-square fits.

The critical depth z_{crit} is the thickness of the thoroughly mixed water column in which the mean light intensity equals I_{comp}. It can be approximated using measured intensities of the photosynthetically active radiation at the water surface (I_0), the mean vertical light attenuation coefficient (ε) and I_{comp} using the Lambert-Beer law as

$$z_{crit} = \frac{I_0}{\varepsilon * I_{comp}} \qquad \text{eq. 3}$$

Differences in the light-growth parameters between experimental treatments were assessed using the nonlinear model given in equation 2. To compare the effects of fluctuating and constant light, we tested the null hypothesis that the model parameters did not vary between the two treatments (fixed depth or vertically moved) against the alternative hypothesis that one or more of the parameters did vary between treatments. Conclusions on treatment effects were based on model comparisons with F-tests according to Bates and Watts (1988, p. 105ff.). Parameters of the photosynthesis curves (α_P, ETR_{max}, I_{kP}) were compared using t-tests. Statistical tests were performed with R version 3.1.3 (R core team, 2015) and SPSS V22.

Results

Mixing conditions and light supply

The near-surface (0-3 m) water temperature increased from 13.3±0.1 °C to 14.6±0.06 °C during our experiments (from the mornings of April 4 to 14, supplemental material, Fig. S1). At the same time, mean temperatures at 10-14 m depth increased from 12.4±0.3 °C to 13.7±0.5 °C. Temperature gradients above 0.5 °C m^{-1} were measured at depths between 11.5 and 15 m in the first experiment and between 10.5 and 13 m in the second one. Weak secondary thermoclines were observed in the afternoons of warmer days: at depths of about 1.5 m on April 4, 8-10 and 13, and at 3.5 m on April 10 and 11. The thermal stratification was always weak, and the squared stability frequency N^2 never exceeded 0.002 s^{-2}. Increased phytoplankton concentrations (measured as chlorophyll fluorescence *in situ*) near the water surface were found in the afternoons of all days except for April 5 and 13, as well as in the mornings of April 4, 6 and 11 (Fig. S1).

Daily PAR at the water surface varied between 2.4 and 31.2 E m^{-2} d^{-1} (Table 1). During the first experiment, one sunny day was followed by one dull and two hazy days. The second experiment was performed in a rather sunny period, with thin cloud cover on the second day and a rainy third day. Vertical light attenuation ranged from 0.91-1.19 m^{-1} (average 0-6 m). The calculated daily PAR intensities in the water column and at the depths of the stationary samples are given in Table 1. Instantaneous PAR in the vertically moved bottles fluctuated by 2-3 orders of magnitude within 20 minutes but remained nearly constant in samples at fixed depths (see Fig. 1 as an example).

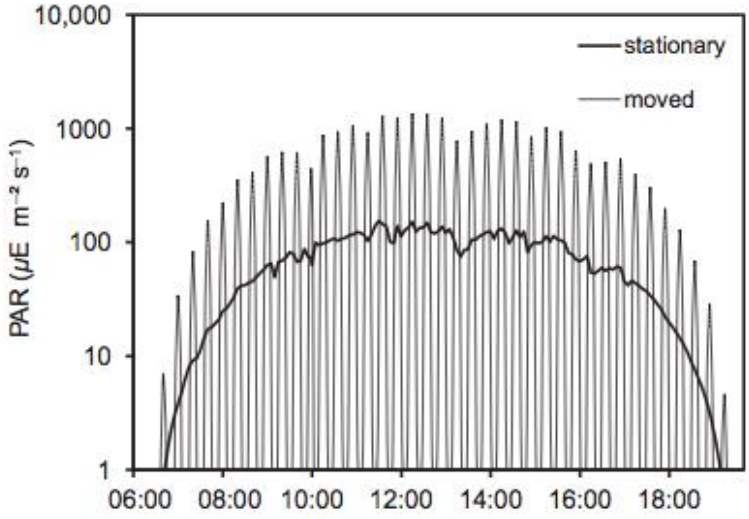


Figure 1. Typical diurnal courses of photosynthetically active radiation experienced by phytoplankton samples moved between the water surface and 7 m depth (fine line), and kept at a fixed depth (1.9 m, thick line), 04 April 2011.

Figure 2 depicts the cumulative frequency of PAR received by algae moved in the upper 7 m and by the respective stationary samples from sunrise to sunset. Even on sunny days, the vertically moved algae spent 60% of the day at PAR below 10 µEm^{-2} s^{-1}. At constant depth, this percentage ranged between 14% on sunny days and 28% on overcast days. On the other hand, the vertically moved algae were also exposed to PAR stronger than 200 µE m^{-2} s^{-1} during 7% of the overcast days and 18% of the sunny days. The corresponding sample at constant depth never received such strong light. On average, mixing shortened the available daylengths (with PAR > 10 µE m^{-2} s^{-1}) by 33±14% (0-3 m), 64±5% (0-7 m), 69±4% (0-10 m) and 72±6% (0-14 m), respectively. On very hazy days (< 1 E $m^{-2}d^{-1}$), phytoplankton at fixed depths spent 39-100% of the period between sunrise and sunset at PAR intensities below 10 µE m^{-2}

s^{-1}. At all higher daily light exposures, this percentage (25.5±8.3%) was significantly lower for stationary samples than for vertically moved samples ($p<0.001$).

Table 1. Photosynthetically active radiation per day at the water surface and received by algal samples which were either vertically moved between the water surface and 3 m, 7 m, 10 m, or 14 m depth, or incubated at respective fixed depths (in E m^{-2} d^{-1}).

		0–3 m		0–7 m		0–10 m/14 m	
Day	Surface	Fixed	Moved	Fixed	Moved	Fixed	Moved
04 Apr	29.58	6.56	10.59	3.28	6.63	1.71	4.29
05 Apr	2.44	0.68	0.96	0.38	0.59	0.23	0.39
06 Apr	10.75	1.87	3.37	0.47	2.33	0.14	1.50
07 Apr	8.19	2.71	3.10	1.07	1.89	0.51	1.23
10 Apr	31.23	10.31	12.58	4.49	7.96	2.96	6.47
11 Apr	18.02	5.17	6.75	2.03	4.30	1.27	3.47
12 Apr	9.94	3.48	3.89	1.48	2.43	0.92	1.98
13 Apr	25.66	8.92	10.32	4.31	6.49	3.10	5.28

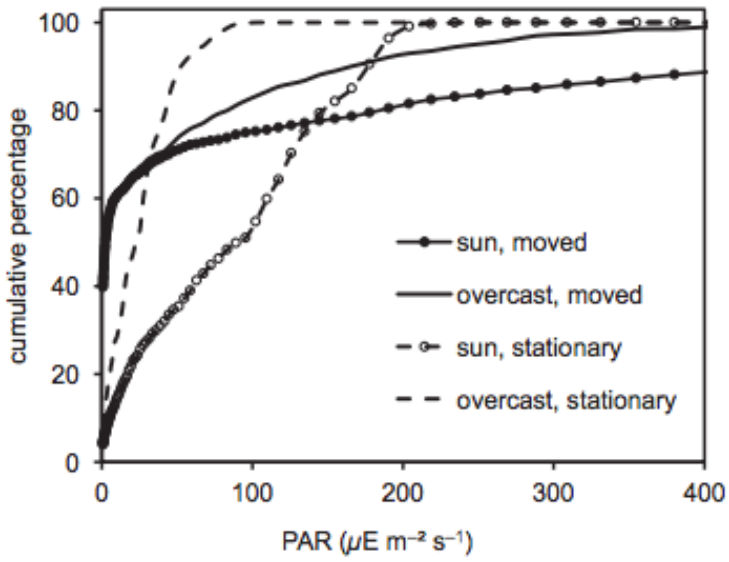


Figure 2. Cumulative percentage of light intensities received by vertically moved (0–7 m; solid lines, filled circles) and by the respective stationary samples (broken lines, open circles). Averages of the sunny (04 April, 10 April, and 13 April; circles) and of the overcast days (06 April, 07 April, and 12 April).

Light dependency of growth

Growth rates increased with increasing global radiation and with declining mixing depth. Growth was saturated in the stationary samples at a daily light supply of 1.18 E m^{-2} ($I_{k\mu}$). The vertically moved algae needed 3.77 E m^{-2} d^{-1} to obtain maximum growth rates (Fig. 3, Table 2). Assuming 12.5 hours daylength, growth was light-saturated at a mean PAR of 26 and 84 µE m^{-2} s^{-1}, respectively. The maximum growth rates μ_{max} did not significantly differ between light regimes (p=0.27). Maximum growth rates averaged at 0.44 ± 0.11 (moved) and 0.38 ± 0.05 per day (fixed depth). At sub-saturating daily PAR, phytoplankton used fluctuating light less efficiently than relatively constant light (p<0.001). The slope of the relation between growth and daily PAR at limiting intensities (α_μ) was calculated as 0.12 ± 0.02 m^2 E^{-1} under fluctuating light and 0.32 ± 0.08 m^2 E^{-1} in fixed depth samples. Accordingly, the compensation light intensity (I_{comp} = daily PAR at zero net growth) was higher for vertically moved than for stationary samples. Photosynthesis and losses were balanced at 0.76 E m^{-2} d^{-1} under relatively constant light (fixed depths) but only at 2.50 E m^{-2} d^{-1} under fluctuating light (moved bottles). These minimum daily light requirements would be equivalent to a mean PAR of about 17 and 55 µE m^{-2} s^{-1}, assuming a 12.5 hours daylength. The difference between I_{comp} and $I_{k\mu}$ was surprisingly small because of unavoidable grazing losses, which affect I_{comp} but not $I_{k\mu}$.

Table 2. Parameters of light-dependency of growth and photosynthesis. Averages, standard deviations, and significance of differences between stationary and vertically moved samples.

Parameter	Unit	Stationary	Moved	*p*
μ_{max}	d^{-1}	0.383 ± 0.053	0.443 ± 0.106	0.27
α_μ	m^2 E^{-1}	0.324 ± 0.080	0.117 ± 0.021	<0.001
I_{comp}	E m^{-2} d^{-1}	0.764 ± 0.126	2.496 ± 0.304	<0.001
$I_{k\mu}$	E m^{-2} d^{-1}	1.18 + 0.39	3.77 + 1.35	<0.001
ETR_{max}	rel. units	46.9 ± 5.1	54.5 ± 8.5	<0.001
α_P	rel. units (µE m^{-2} s^{-1})$^{-1}$	0.267 + 0.045	0.278 + 0.029	0.065
I_{kP}	µE m^{-2} s^{-1}	183 ± 51	199 ± 46	0.047

The high I_{comp} of vertically moved phytoplankton resulted in critical depths between near-zero on a dull day and 13.9 m on a sunny day (Fig. 4). I_{comp} of samples at fixed depths was about 69% lower, and accordingly the critical depths were higher (3.1 m - 45 m, depending on daily global radiation and underwater light attenuation). On dull days (April 5-7), all approaches resulted in critical depths above the thermocline.

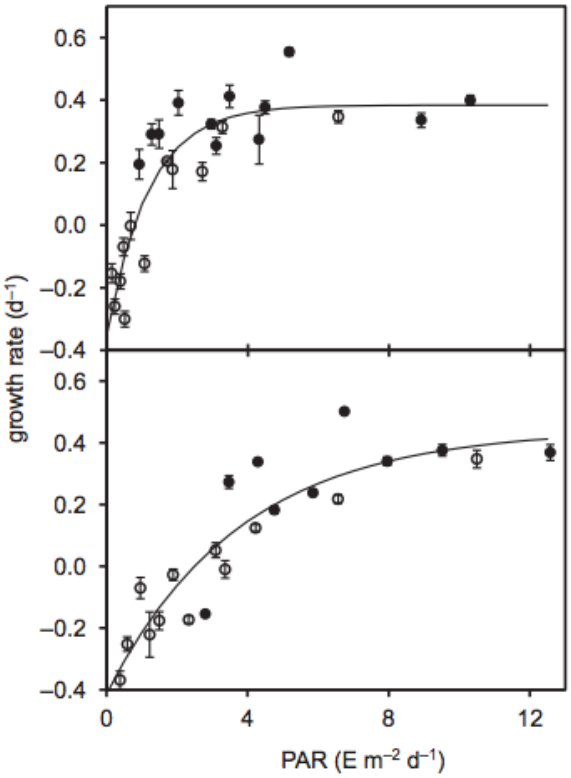


Figure 3. Light-dependency of growth of phytoplankton incubated at constant depth (top) and vertically moved (bottom), mean growth rates and standard deviations. Open symbols: 04–08 April, filled symbols: 10–14 April. Solid lines depict the model fits (Eq. 2).

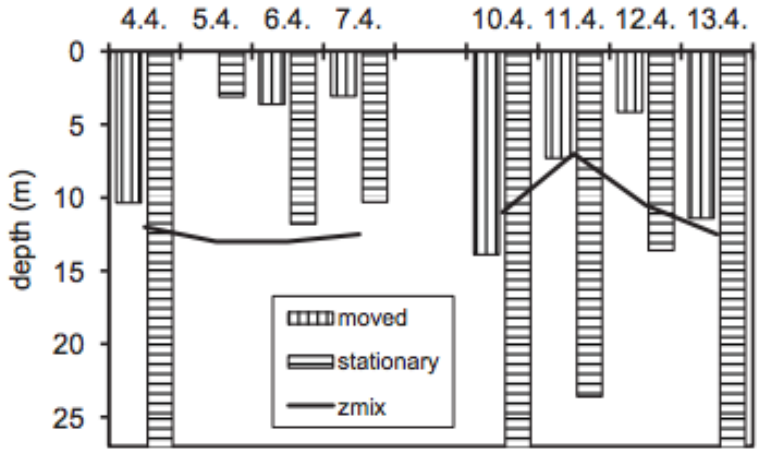


Figure 4. Critical depths and mixing depths during the experiments (04–13 April). Critical depths were calculated using Icomp obtained from growth experiments with vertically moved (vertical lines) or stationary samples (horizontal lines).

Photosynthesis

The maximum relative electron transport rates were on average higher after mixing than after stagnant conditions (Table 2). ETR_{max} increased with increasing mixing depth, from 47 (0-3m) to 55 (0-7m) to 61 rel. units (0-10/14m) but did not significantly change with depth under stagnant conditions. There was no significant difference between moved and fixed samples near the surface (0-3 m) but ETR_{max} was higher in bottles moved between the surface and 7m or deeper than in the respective bottles at fixed depth. Photosynthesis was usually saturated at higher PAR intensities I_{kP} (= ETR_{max} / α_P) in moved samples than in stationary samples (Table 2). The only exception was the near-surface (0-3 m) sample during the first run. Photosynthesis was saturated at much higher light intensities than growth (I_{kp} > $I_{k\mu}$). The photosynthetic efficiency at sub-saturating light (α_P) did not significantly differ between depths or treatments.

These photosynthesis-light parameters and the diurnal courses of underwater light intensities were used for modeling of the diurnal ETR. Near the water surface, instantaneous PAR exceeded I_{kp} for most of the time on sunny days. Accordingly, photosynthesis of vertically moved algae approached ETR_{max}, which implies a lowered photosynthetic efficiency (ETR / PAR) during their stay in upper water layers (see Fig.5 as an example). The ETR of the respective stationary samples never reached this upper limit; their photosynthesis mostly operated at maximum efficiency. The mean ETR per revolution in moved samples was lower

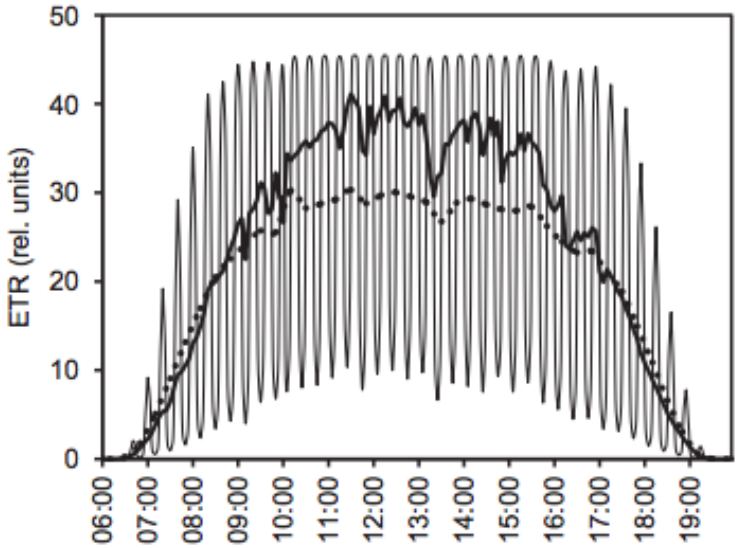


Figure 5. Diurnal courses of photosynthesis (in relative ETR) of phytoplankton at 1.3 m depth (thick solid line) and moved between water surface and 3 m depth (thin solid line; the dots illustrate the averages per revolution), 04 April 2011.

than that of the respective stationary sample during most of the day (from about 9:30 to 16:30). The relations between modeled daily production and daily light supply are given in Fig. 6. Here, the same set of parameters (from stationary samples) was applied to both modes of light dynamics to quantify the effect of the different light distribution. The fitted daily maximum ETR was similar (p=0.94) but α_p per day was 47% lower for vertically moved (0.129 rel. units) than for stationary algae (0.243 rel. units; p<0.0001).

Discussion

Maximum growth rates

The effects of fluctuating light on algal growth most probably depend on the range of light intensities received. At high surface irradiance and low optical mixing depth ($\varepsilon \cdot z_{mix}$), planktonic algae may receive growth-saturating light intensities in the largest part of the mixed water column. Under such conditions, algae transported over moderate vertical distances should grow at the same maximum rates as algae residing at an optimum depth. Such low optical mixing depths are typically found in clear waters (ocean, oligotrophic lakes)

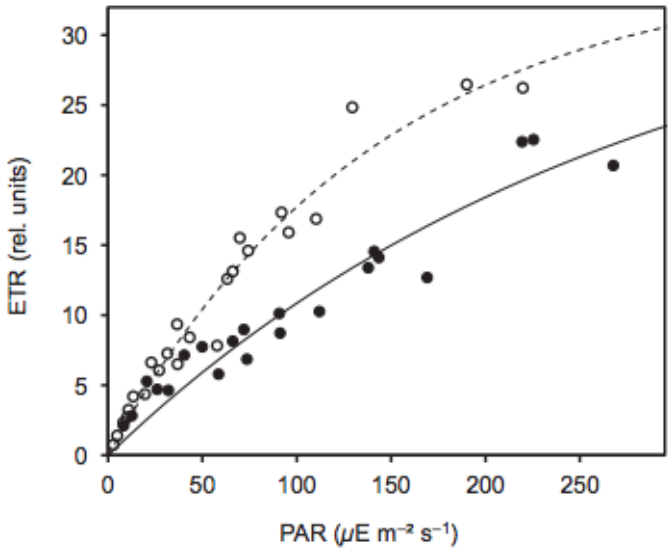


Figure 6. Light-dependency of daily production (in relative ETR). Daily production was integrated from photosynthesis calculated every 75 s using the parameters of the photosynthesis-light relation of stationary samples and the PAR available to either vertically moved (filled circles) or stationary samples (open circles). Lines indicate the model results (Eq. 2).

with shallow mixing layers, *e.g.* at the beginning of thermal stratification or on calm days, and in shallow waters of low to moderate turbidity (e.g., slightly eutrophic shallow lakes or rivers). In our experiment, such conditions occurred on the two days with the highest global radiation (April 4 and 13) in the near-surface layer (0-3m) with z_{eu}:z_{mix} ratios of 1.32 and 1.67, respectively. There, both stationary and vertically moved algae received saturating PAR for more than 70% of the day (Fig. 7a), spent about 20% of the day in effective darkness (Fig. 7b) and attained similar maximum growth rates. Litchman (2000) and Dimier et al. (2009) also found no significant influence of light dynamics on growth rates if light intensities always exceeded $I_{k\mu}$. Nicklisch & Fietz (2001) and Shatwell et al. (2012) simulated deeper mixing under lab conditions and found lower μ_{max} at fluctuating than at constant light. The difference increased with declining z_{eu}:z_{mix} ratios (or shorter effective daylength). In the latter experiment, phytoplankton spent 25% of the day with PAR <10 µE m^{-2} s^{-1} at z_{eu}:z_{mix} = 1 and 58% of the day at z_{eu}:z_{mix} = 0.5 whereas the respective percentages ranged between 2.6% and 3.1% under constant light of the same daily intensity (8.3 E m^{-2} d^{-1}).

Near the water surface, phytoplankton may be exposed to inhibiting light intensities, mostly due to ultraviolet radiation (*e.g.* Cullen et al. 1992). The effects of strong light exposure on algal growth are dosage-dependent (*e.g.* Marra 1978). Algae can repair effects of short term exposures but suffer permanent damage if inhibiting light intensities last too long. Repair mechanisms are most efficient at low light (Anderson et al. 1997). Therefore, turbulent mixing may mitigate inhibition of photosynthesis (Ibelings et al. 1994) but this effect depends, among other factors, on the z_{eu}:z_{mix} ratio (Neale et al. 1998, Köhler et al. 2001, Barbieri et al. 2002). The Duran glass bottles used for our incubations absorbed more than 90% of UV-B and about 50% of the radiation at 340 nm (Köhler et al. 2001). Therefore, photoinhibition was unlikely in our experiment but it may favour vertically moved algae over algae residing near the water surface on bright days. Without this incubation effect, the maximum growth rate under fluctuating light may exceed that under constant light of the same mean intensity.

Growth efficiency

In our experiment, vertically moved algae grew more slowly than algae at constant depth of equivalent sub-saturating daily PAR. Again, the different distribution of light intensities probably caused these differences in growth rates: Already at 7m mixing depth, the vertically

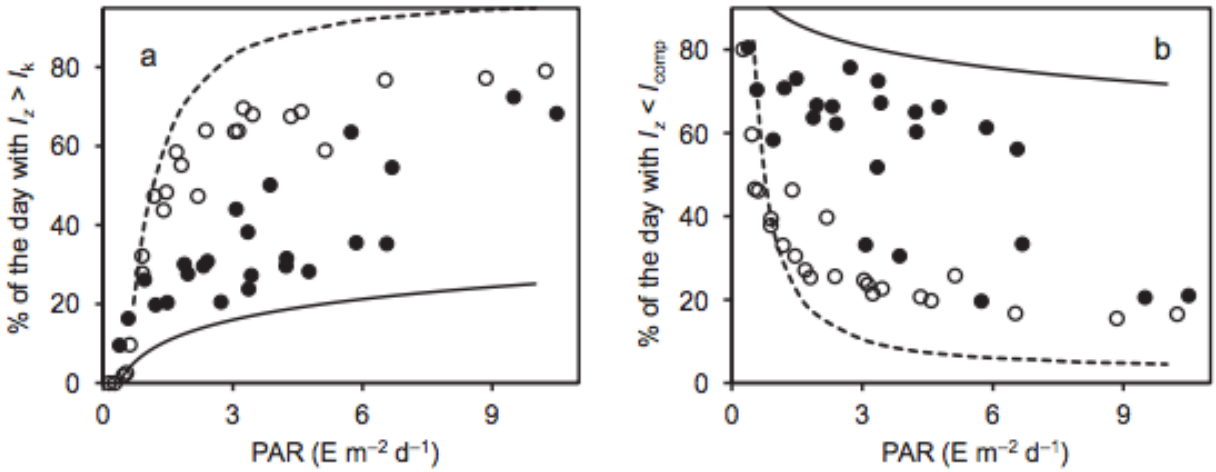


Figure 7. Percentage of the day with (a) saturating light intensities (> 26 µE m^{-2} s^{-1}) and (b) in the aphotic zone (< 10 µE m^{-2} s^{-1}) vs. daily light supply. Here, the same thresholds were set for both modes to facilitate comparability. Circles indicate measured data and lines the model results (see text for explanation). Open circles and broken lines: stationary samples, filled circles and solid lines: vertically moved samples. The model assumes that the diurnal course of global radiation follows a sine curve whereas the real light intensities often fell below this optimum.

moved algae spent two to four times longer at an instantaneous PAR below 10 µE m^{-2} s^{-1} than their stationary counterparts (Fig. 2). The shorter effective daylength available to vertically moved algae results in decreased growth rates (Boelen et al. 2011, Shatwell et al. 2012, Hoppe et al. 2015). Vertically moved algae also received saturating light during longer parts of the day than the stationary algae (Fig. 2). Light intensities above $I_{k\mu}$ increased the mean daily light supply but not the growth rate. Accordingly, the higher percentage of saturating light may explain lower growth rates under fluctuating than under constant light of the same intensity found by van de Poll et al. (2007). Nicklisch & Fietz (2001) and Shatwell et al. (2012) compared growth rates at several mean intensities of constant and fluctuating light. Light fluctuations reduced growth efficiency α_μ of *Planktothrix agardhii*, *Stephanodiscus neoastraea* (Nicklisch & Fietz 2001) and *Limnothrix redekei*, but not of *Stephanodiscus minutulus* or *Nitzschia acicularis* (Shatwell et al. 2012).

The lower growth efficiency implies a higher daily light demand $I_{K\mu}$ to saturate growth under fluctuating light. Interestingly, growth saturated at much lower light intensities than photosynthesis. In our study, electron transport rates of stationary and of vertically moved phytoplankton saturated at 183 and 199 µE m^{-2} s^{-1} whereas growth saturated at a mean PAR of 26 and 84 µE m^{-2} s^{-1}, respectively (at 12.5 hours daylength). Shatwell et al. (2012) found

electron transport rates of diatom and cyanobacteria cultures saturated at a PAR between 182 and 289 µE m^{-2} s^{-1} whereas growth saturated at 24-44 µE m^{-2} s^{-1} (daily average). Similar differences were found for the cyanobacterium *Limnothrix redekei* by Gibson & Foy (1983). Stagnant growth but still increasing photosynthesis at light intensities between $I_{K\mu}$ and I_{KP} is explainable only by an increase of physiological losses with increasing light. Indeed, the few available studies indicate higher rates of respiration (Grande et al. 1989, Luz et al. 2002) and exudation (Zlotnik & Dubinsky 1989, Maranon et al. 2004) in the light compared to the dark.

Compensation light intensity and critical mixing depth

Almost all estimates of I_{comp} are based on measurements of growth (Hobson & Guest 1983, Falkowski et al. 1985) or photosynthesis and losses (Langdon 1988) under constant light. In stratified water columns, phytoplankton may adapt to relatively constant low light to form distinct deep chlorophyll maxima. Adaptive strategies involve the reduction of metabolic maintenance costs (*e.g.* lower dark respiration) and increased photosynthetic efficiency (*e.g.* higher absorption cross section, higher ratio of photosynthetic to protective pigments, see review of Dubinsky & Stambler 2009). Some species adapted to permanently low light may grow at a mean PAR of 1-2 µE m^{-2} s^{-1} or 0.05-0.1 E m^{-2} d^{-1} (*e.g.* Geider et al. 1985, Bright & Walsby 2000). Marra et al. (2014) estimated zero daily net carbon assimilation of phytoplankton samples kept at water depths with a daily PAR of about 0.1-0.2 E m^{-2}. Laboratory experiments under constant low light found zero growth at light intensities in the range of 0.1 to 0.8 E m^{-2} d^{-1}, with the exceptions of higher I_{comp} for dinoflagellates (Langdon 1988) or chlorophytes (Richardson et al. 1983). Our phytoplankton samples incubated at constant depths needed about 0.77 E m^{-2} d^{-1} to balance production and losses. This I_{comp} value ranges at the upper end of the published data, probably because of additional losses in our samples (*e.g.* grazing by microzooplankton) compared to experiments with algal cultures (see Nelson & Smith 1991).

Only very few compensation light intensities were experimentally determined under fluctuating light. The laboratory study of Nicklisch & Fietz (2001) indicated I_{comp} close to zero regardless of the light regime. Gibson (1985) measured I_{comp} of 0.1-0.2 E m^{-2} d^{-1} in short on-off cycles of saturating light but this is hardly comparable to natural light fluctuations. On an ecosystem level, a mean radiation of about 0.03 cal cm^{-2} min^{-1} (or about 1.9 E m^{-2} d^{-1}) in the water column was critical for initiation of spring development of phytoplankton in coastal waters (Riley 1957). Siegel et al. (2002) estimated I_{comp} as mean light intensity in the mixed

surface layer at the start of the spring development of phytoplankton in the North Atlantic. This approach gave a mean I_{comp} of 1.0-1.7 E m^{-2} d^{-1} in large parts of the ocean. In our "yo-yo" experiment, phytoplankton communities needed about 2.5 E m^{-2} d^{-1} to compensate losses. In accordance with our findings, the few published relevant field studies indicate much higher minimum daily light requirements of phytoplankton under mixing conditions than for algae adapted to constant low light. Again, this difference is probably caused by the much longer part of the day spent at very low light intensities under mixing than under stagnant conditions. For instance, at z_{mix}=7m, vertically moved algae spent about 50% of the day at light intensities below 2 µE m^{-2} s^{-1} whereas this percentage ranged between 6 and 12% for stationary algae (Fig. 2).

The compensation light intensity is crucial for calculations of the critical mixing depth z_{crit}, the depth of the surface mixing layer with a mean light intensity approaching I_{comp}. Under nutrient-replete steady-state conditions, phytoplankton grows until self-shading reduces the mean light intensity in the mixing layer to I_{comp}. Therefore, estimates of z_{crit} are as precise as I_{comp}. As was demonstrated in our experiment, the estimation of I_{comp} under invariable light seriously underestimates minimum light requirements of phytoplankton in mixed water layers. Accordingly, it overestimates the critical mixing depth. In our experiment, z_{crit} was often smaller than z_{mix} (Fig. 4), suggesting a dominance of loss processes in such periods. However, z_{mix} was, as usual, estimated from vertical temperature gradients. Potentially, the upper mixed layer was not turbulent enough to homogeneously distribute the phytoplankton (see Franks 2015). Below a critical turbulence, growth rates may exceed rates of vertical transport, enabling phytoplankton growth irrespective of z_{mix} (Huisman et al. 1999).

Effects of nonlinearity vs. effects of acclimation

The frequency distribution of underwater light can be generalized mathematically in terms of the mean daily light to which algae are exposed (I_{mean}). At fixed depth, the proportion of the day f that algae spend below instantaneous light intensity I, assuming that incoming radiation follows a sine curve during the day, is

$$f(I) = \frac{2}{\pi} \sin^{-1}\left(\frac{2ID}{\pi I_{mean}} \right)$$

where D is the solar daylength as a fraction of a 24-hour day. Accordingly, algae at fixed depth spend $f(I_{comp})$ at subcompensation intensities and 1-$f(I_{k\mu})$ at supersaturating intensities

(see lines for fixed samples in Fig. 7). Under well-mixed conditions, the proportion of the water column with intensity greater than I is z_I/z_{mix}. (assuming $0 < z_I < z_{mix}$), where z_I is the depth of intensity I:

$$\frac{z_I}{z_{mix}} = \frac{\ln\left(\frac{I_0}{I}\right)}{\varepsilon z_{mix}}$$

Considering that I_0 varies over time (t in days), the proportion of the day algae spend above I is given by integrating over t as $\int_0^D z_I z_{mix}^{-1} dt$ (see lines for moved samples in Fig. 7). Therefore as shown in Fig. 7, stationary samples spend a greater part of the day above compensation intensities than moved samples. Moreover, stationary samples are exposed longer to intensities between I_{comp} and $I_{k\mu}$, which can be used most efficiently, and this amount of exposure increases relative to moved samples as mean daily light supply decreases. This helps to explain why, when averaged over a day, vertically moved samples grew more slowly at low light, but no difference was observed at high daily light.

In order to estimate the effect of different frequency distributions of light intensity, production rates were calculated at a temporal resolution of 75 secs using the photosynthesis-light parameters of stationary algae for both modes and the instantaneous light intensities experienced by vertically moved or by static samples (Fig. 5). The daily integrals of production indicated a 47% lower efficiency of vertically moved than of stationary algae (Fig. 6). According to the measured daily growth rates, α_μ was 64% lower under fluctuating than under constant light (table 2). In other words, roughly three quarter of the found gap in growth efficiency between vertically moved and stationary algae can be attributed to the different frequency distribution of light intensities, *e.g.* the higher percentage of less efficiently used saturating light under mixing. This comparison confirms our first hypothesis, even though it provides rough estimates rather than exact numbers. The approach could be further improved by taking the diurnal course of photosynthesis-light parameters into account. If the photosynthetic electron transport saturates at higher PAR than carbon assimilation (*e.g.* Hancke et al. 2015) the fluorometric method used would overestimate I_{kp} and thus slightly underestimate the effect of nonlinearity in the photosynthetic response to fluctuating light.

The remaining quarter of the efficiency gap should be caused by light-dependent losses or by imperfect acclimation to fluctuating light. At the time scale of Langmuir cells, phytoplankton can acclimate to light fluctuations by state-transitions (Falkowski et al. 1994) and changes in the activation state of Rubisco (MacIntyre et al. 2000). The xanthophyll cycle is another important short-term light acclimation mechanism in diatoms and chlorophytes, but is not possessed by cyanobacteria or cryptophytes (*e.g.* Demming-Adams & Adams 1996). The interplay of an orange carotenoid protein and the phycobilisome can regulate photosynthesis vs. energy quenching in cyanobacteria (Kirilovsky & Kerfeld 2016). Under natural conditions, movement of phytoplankton is certainly less constant. Turbulent mixing may cause more irregular light fluctuations which require even faster acclimation.

These mechanisms are based on assembly of enzymes or pigments or on dissipation of absorbed energy. They inevitably reduce the efficiency of conversion of irradiance into biomass compared to constant light of the same mean intensity (*e.g.* Su et al. 2012). Energy requirements of acclimations should be more relevant under limiting than under saturating light supply. Accordingly, dynamic irradiance should affect growth efficiency at sub-saturating light α_μ more than maximum growth at saturating light μ_{max}, as was observed in this study.

On the other hand, fluctuating light may force acclimation to stronger light intensities in order to avoid damage to the photosystems and to better exploit bright light near the surface. The acclimation to light intensities higher than what is on average available is advantageous only under mixing conditions (Cullen & MacIntyre 1998). Such acclimation explains the higher maximum rates of photosynthesis under mixing than under stagnant conditions, as were found in our study (Table 2). This difference was probably even underestimated in our measurements after dark adaptation.

Conclusions

The present study provides evidence for substantial effects of vertical mixing on compensation light intensity and on growth efficiency of phytoplankton at sub-saturating light. The decline in growth-efficiency under vertical mixing was largely caused by the nonlinear light-dependency of photosynthesis and growth. This part of the mixing effects can be calculated if the frequency distribution of the light received by the mixed algae is known.

The remaining gap in growth efficiencies can be attributed to (species-specific) acclimation mechanisms and to light-dependency of physiological losses. The dynamics of these processes requires more simultaneous studies of physiology and turbulence-driven vertical movement of planktonic algae. This would allow a better understanding and prediction of the effects of mixing on phytoplankton development.

Acknowledgements - The experiments were conducted at the field station of the Institute of Hydrobiology, Wuhan, of the Chinese Academy of Sciences. We thank Qinghua Cai for providing these facilities and Lu Tan and Qiande Yuan for measuring vertical profiles of light, temperature and chlorophyll fluorescence. Phytoplankton was identified and counted by Wolfgang Arp. The Sino-German Center for Research Promotion (DFG/NFSC) covered the travel expenses of JK (GZ 465). AG was funded by the German Federal Ministry of Education and Research (02WCL1336D). We thank Patrick Neale and an anonymous reviewer for helpful suggestions.

Chapter 2

Manuscript title:

Interplay between photosynthesis, respiration and growth of phytoplankton communities under vertical mixing.

Alexis Guislain[1,2*] and Jan Köhler[1]

[1]Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Department of Ecosystem Research, Berlin, Germany.

[2]University of Potsdam, Department of Ecology and Ecosystem Modeling, Potsdam, Germany.

Under review in Frontiers in Freshwater Science.

Abstract

Photosynthesis and growth of suspended algae (phytoplankton) depend on the mean intensity and temporal distribution of light. Available light fluctuates greatly over time and space in the mixing layer. Mixing algae face a tradeoff between resource allocation to photo-protective mechanisms at strong near-surface light and efficiency of photosynthesis and growth at limiting light intensities. The interplay between photosynthesis, respiration and growth is largely unknown under such dynamic conditions. We hypothesise a reduced growth efficiency of deeper mixing phytoplankton by shortened effective daylength and enhanced respiration. In contrast, inefficient protection to inhibiting light intensities of surface algae should increase the maintenance respiratory costs, lead to photosynthesis foregone during photorepair and eventually reduce growth rates. Till now, effects of fluctuating light on phytoplankton were usually studied on monocultures in the laboratory after a period of light acclimation. In the present study, phytoplankton communities of the well-mixed, hypertrophic Lake TaiHu (China) were incubated in situ either at fixed depths or at simulated vertical mixing. Growth, photosynthesis, production and night respiration were measured.

At sub-saturating daily light intensities, mixing algae grew 39 % less efficient compared to constant light conditions. About 1/3 of this decline was attributed to shortened effective daylength for gross oxygen production as mixing algae spent longer periods in effective darkness. The remaining difference could be attributed to higher losses for biosynthesis in effective darkness and acclimation to fluctuating light supply. The mixing algae did not only produce less, they also respired less at saturating light than the stationary algae. Compared with fixed algae, mixing algae harvested and exploited surface irradiance more effectively by enhancing potential photosynthesis while avoiding photo-inhibition and likely rapidly enhancing respiration when located in effective darkness. Our observations contribute to a better understanding of community eco-physiology and more reliable predictions of phytoplankton development under vertical mixing.

Keywords: Fluctuating light, Effective daylength, Growth efficiency, Oxygen production, Photo-inhibition, Photosynthesis, Respiration, Lake TaiHu.

Introduction

As the major primary producers on Earth, phytoplankton are responsible for about half of the global net production of photosynthetic organisms (Field et al., 1998) and therefore greatly affect food webs and biogeochemical cycles (Falkowski et al., 1998; Litchman et al., 2015). Phytoplankton growth depends on the intensity of photosynthetically active radiation (PAR), which also influences the temperature (Edwards et al., 2016) and the nutrient dependency of growth (Litchman et al., 2004a). Light is a highly dynamic resource (Falkowski, 1984; Ferris and Christian, 1991). In nature, global radiation fluctuates according to seasons, diurnal changes as well as cloud cover. In the water column, light decreases exponentially with increasing optical depth (product of depth and vertical light attenuation). Superimposed, suspended algae experience light of fluctuating intensity during transport in the mixing layer (Kirk, 1994).

Photosynthesis and growth depend nonlinearly on light supply. Thus, they depend on the mean light intensity and also on the temporal light distribution (Litchman, 2000). If algae are transported below their compensation light intensity for growth, the effective daylength is shortened and may reduce growth rates (Boelen et al., 2011; Hoppe et al., 2015; Shatwell et al., 2012). Also, because of this nonlinear response of growth to light, growth should be less efficient when the light supply fluctuates between very low and saturating intensities compared to when constant and sub-saturating at the same mean intensity (Thornley, 1974; Dromgoole, 1988; Litchman, 2000). Köhler et al. (2018) estimated that different frequency distribution of underwater light was responsible for about three quarter of the significant decline in growth efficiency at sub-saturating light of a lake community experiencing vertical mixing compared to algae incubated at fixed depths. The remaining quarter was expected to be due to losses and acclimations to fluctuating light supply of on average limiting intensity. Light fluctuations affect several physiological processes such as photosynthesis (Fietz and Nicklisch, 2002), respiration (Beardall et al., 1994), exudation (Cosper, 1982) and consequently, growth (Guislain et al., 2019a; Shatwell et al., 2012). Photoacclimation to fluctuating light is species (potentially even clonal-dependent, Kardinaal et al. 2007) and timescale dependent (Litchman, 2000; Macintyre et al., 2000). Vertically mixed algae have to deal with extreme light changes and therefore have been shown to preferably enhance their photosynthesis to benefit from rapid surface light peaks while investing a significant amount of energy into protection against light inhibition (Dubinsky and Stambler, 2009; Talmy et al.,

2013). On the contrary, algae receiving relatively constant low light intensities would generally allocate more energy into an efficient light harvesting machinery (*e.g.* high chlorophyll : C ratio) but may be growth saturated at lower light intensities. Overall, there is a tradeoff between resource allocation to mechanisms that protect against strong light and efficiency of photosynthesis and growth at low light intensities (MacIntyre et al., 2002; Talmy et al., 2013). Inefficient protection to inhibiting light intensities increases energy costs of repair (*i.e.* for protein D1 of the Photosystem II, Ferris and Christian 1991 for review). Besides photosynthesis foregone during photorepair, the photo-damage would require more respiratory costs for protein synthesis and ultimately impair resource allocation to growth (Raven, 2011).

Most of the studies on effects of fluctuating light on phytoplankton used monocultures, were performed in the laboratory and allowed a period of acclimation of algae to a certain light condition before measurements (Graff et al., 2016; Nicklisch, 1998; Shatwell et al., 2012). Nonetheless, constant conditions rarely occur in ecosystems (Falkowski, 1984; Longhi and Beisner, 2009). Photosynthesis and respiration are essential for biosynthesis and are dynamic responses of mixing algae to mean and temporal distribution of irradiance at vertical mixing (Ferris and Christian 1991 for review). However, previous investigations on the effects of fluctuating light mostly focused on either growth, photosynthesis or physiological losses. The present study aims at better understanding the understudied interplay between the light-dependent photosynthesis, respiration and growth of phytoplankton communities under fluctuating light exposure.

We hypothesized that in a lake phytoplankton community:

H1. At saturating light intensities: extended exposure of surface algae to inhibiting irradiances leads to decreased production, higher respiratory maintenance costs and lower community growth rates. On the contrary, photo-inhibition should not occur under mixing conditions. Photosynthesis enhancement should be observed and we expect higher growth rates of mixing algae than under constant high light exposure.

H2. At sub-saturating light intensities: at the same daily irradiance, mixing algae should stay for longer parts of the day at light intensities below the compensation light intensity and above the onset of saturation than algae at fixed depths. Also, mixing algae should take advantage of periods in effective darkness to rapidly trigger respiration. Nonlinearity, shorter

effective daylength and higher respiration rates should reduce production and growth per light supply of mixing compared to stationary algae.

To test these hypotheses, we mimicked vertical mixing and induced fluctuating light regimes by computer-controlled motion of subsamples from a phytoplankton community in the frequently mixed, turbid, hypertrophic Lake TaiHu (China). For comparison, subsamples were incubated at four fixed depths. The investigated community was adapted to Lake TaiHu's temperature and frequent mixing. It was incubated under nutrient-replete conditions and drastically reduced grazing pressure. We analyzed photosynthesis, gross and net oxygen production, night respiration and community growth.

Material and Methods

Study site and abiotic conditions

Lake TaiHu (China, 31°14'N 120°8'E) is a very large (2340 km^2), shallow (1.9 m mean depth), hypertrophic, turbid and wind-exposed lake (Duan et al., 2009; Qin et al., 2010). The field experiment was conducted from September 7th to September 16th 2016, during the development of a cyanobacteria bloom (mostly *Microcystis spp*., see Guislain et al., 2019). Our experimental site was located in the Meiliang Bay (northern part of Lake TaiHu) on top of the Nanjing Institute of Geography and Limnology (NIGLAS) landing, about 200 meters offshore.

Global radiation data were measured using a 2π light sensor type and were obtained from the NIGLAS monitoring station (TaiHu Laboratory for Lake Ecosystem Research TLLER) located near the experimental site. To obtain daily PAR exposures, we first calculated the vertical PAR gradient following Lambert-Beer's law:

$$\mathrm{I_z} = \mathrm{I_0} * \mathrm{e^{-kz}}$$

where I_z is the light intensity at depth z (m), I_o is PAR at the water surface and k the light attenuation coefficient (m^{-1}). The latter was calculated from daily light measurements at 0.5 m intervals from the surface to 1.5 m depth with a spherical spectroradiometer (RAMSES-ASC-VIS, TriOS, Germany). Then, we corrected the light data for shade produced by the pier

(when applicable), for wavelength-specific transmittance of the incubation bottles and the actual vertical position of the moved phytoplankton.

Vertical profiles of temperature were measured every 5 minutes using temperature loggers (Aquatic 2 TG-4100, Tinytag, United Kingdom) attached to the bottles holders. The lake was very well mixed with temperatures between the lake surface and the bottom differing by less than 0.36 °C on average during the experimental period.

Experimental setup

Prior to sampling, we removed surface scum containing dying cyanobacteria cells and sampled lake water at 30 cm depth to recover the lake phytoplankton community. We filtered the water through a 100 µm sized mesh to remove large zooplankton, and then gently bubbled it with N_2 for five hours to kill any small remaining zooplankton by anoxia. We added 12× concentrated MIII-KS fresh culture medium, to obtain 1× final concentration (see Nicklisch et al., 2008 for detailed composition). After re-aeration we siphoned the lake water into 500 mL transparent incubation bottles (Teflon Fluorinated Ethylene Propylene, mean transmittance to PAR [400-700 nm] = 72 ± 6.6 % and to UV-A [320-400 nm] = 51.4 ± 3.5 %).

Bottles were installed in triplicates in transparent holders placed at fixed depths and vertically moved by a computer-controlled lift in the lake (Nixdorf and Behrendt, 1991; Köhler et al., 2018). Phytoplankton incubated at constant depth received only the natural sinusoidal diurnal course of sunlight, a treatment that we will refer to as constant light. In contrast, communities incubated in bottles moved vertically through the water column received fluctuating light, by superimposing the vertical light gradient on the natural sinusoidal diurnal sunlight. The lifts simulated a circular movement with 20 minutes per revolution, replicating to some extent the full overturn of typical Langmuir cells (Denman and Gargett, 1983; Schubert and Forster, 1997; Thorpe, 2004). We fixed incubation bottles at 0, 20, 40 and 80 cm depth (fixed samples F0, F20, F40 and F80). The moving bottles rotated between the water surface (0 cm) and 50, 100 and 180 cm depth (lifted samples L50, L100 and L180). The daily PAR doses received in all treatments are given in the Appendix (Appendix - Table 1, Fig. 1).

Subsamples were taken each morning before sunrise. Sample volumes ranged from 80 to 100 mL to ensure similar total biomass (and self-shading) between the different incubation bottles. To avoid nutrient limitation, we refilled the bottles with a mix of filtered lake water (Whatman GF/F glass microfiber) and 12x concentrated MIII-KS fresh culture

medium, to obtain 1x final concentration. The bottles were re-incubated in the lake within 20 minutes. During night, all bottles were placed at 1 m depth.

More details on the experiment setup and the species composition of phytoplankton are available in Guislain et al. (2019).

Phytoplankton biomass

After at least 30 minutes dark adaptation in opaque bottles, three subsamples per bottle were taken to measure the minimal fluorescence of photosynthetic pigments at four wavelengths in a PHYTO-PAM fluorometer (Walz, Germany). The intensities of dark-acclimated minimal fluorescence were converted into chlorophyll *a* concentrations (Chl *a*, in µg L^{-1}) by calibration with Chl *a* measured by high-pressure liquid chromatography HPLC ($n = 93$, $p<0.001$, $R^2 = 0.98$). The HPLC method is detailed in Shatwell et al. (2012).

In parallel, absorbance at 380-760 nm of dark-adapted subsamples was measured directly on three different Whatman GF/F filters per bottle in a spectrophotometer (UV-2450, Shimadzu, Japan). Data were corrected for absorbance of the glass microfiber filter, filter effects (Köhler, unpubl.: filter effect corrected absorbance = 0.1195*absorbance^2 + 0.229*absorbance) and inorganic particles (bleached filter with NaOCl, 1 % free chlorine). Absorbance at 675 nm (average 665-685 nm) was converted into Chl *a* by calibration with HPLC data ($n = 93$, $p<0.001$, $R^2 = 0.98$). Finally, the minimal fluorescence-based and the absorbance-based biomass proxies were averaged. The biomass was linearly related to the microscopically determined total biovolume of species (see Guislain et al., 2019; $n = 32$, $p<0.001$, $R^2 = 0.91$). The fits did not differ significantly between algae incubated at constant or fluctuating light ($p>0.05$).

The absorption cross-section a* (m^2 mg^{-1} Chl *a*) of phytoplankton was calculated as follows:

$$a^* = \frac{A * S * 2.3}{V * \text{Chl } a}$$

where *A* is the average absorption of phytoplankton between 400 nm and 700 nm, *S* is the clearance area of the filter (in m^2), *V* is the filtered volume in mL, *Chl a* is the chlorophyll *a* concentration (mg mL^{-1}) and 2.3 is the conversion factor from $\log_{10}$ to ln.

Photosynthesis

Rapid photosynthesis-light curves were measured directly after quantifying the minimal fluorescence with the PHYTO-PAM. Chl *a*-specific relative electron transport rates (ETR) were measured at 11 increasing light intensities (1-600 µE m^{-2} s^{-1}) after 30 seconds acclimation to each light step.

Net oxygen production and night respiration rates

Oxygen concentrations were recorded continuously in a single bottle per depth (fixed and mixed) every 15 seconds during the day and every 5 minutes during the night using sealed optodes (DP-PSt3, PreSens, Germany) and a 10 channels fiber optic oxygen transmitter (OXY-10, PreSens, Germany). Oxygen concentrations were corrected for temperature (correction provided by PreSens) and oxygen diffusion of the incubation bottles (feature provided by the manufacturer) and then biomass normalized.

The daily net oxygen production rates were calculated from the difference in oxygen concentrations between sunrise and sunset and related to the daily PAR (E m^{-2} d^{-1}) (Appendix - Table 1). The net oxygen production rates were also analyzed for all samples on a 20 min timescale for a day (sunrise to sunset) with low (September 14th) and high daily PAR (September 9th). The 20 min step corresponded to a full cycle of the lifted samples. For consistency, we applied this timescale for the fixed samples as well. Net production rates were calculated from changes in mean oxygen concentrations (averaged for 20 minutes periods) and related to the mean PAR (µE m^{-2} s^{-1}) received by algae during this period (Appendix - Fig. 2).

The night respiration rates were calculated from oxygen changes overnight (excluding periods around sample handling in the evening and just before sunrise) and related to growth rates.

To obtain reliable trends along the light gradient and improve parameter estimation of the effects of light fluctuations on net oxygen production and night respiration, we opted for measuring oxygen concentrations in more samples along the light gradient over more replicates at fewer light intensities. This strategy is in line with the recent call for “regression-based experimental designs” expressing the need to increase the number of predictor levels while decreasing the number of replicates (Beier et al., 2012; Cottingham et al., 2005; De Boeck et al., 2015; Schweiger et al., 2016).

Calculations and statistics

Daily growth rates μ (d^{-1}) were calculated for each sample as follows:

$$\mu = \ln\left[\frac{\text{biomass}_{t1} * \text{vol}}{\text{biomass}_{t0} * (\text{vol} - \text{vol}_{\text{dilution}})}\right]$$

with $biomass_{t0}$ and $biomass_{t1}$ being the community biomass at times t0 and t1. *Vol* is the total volume of the incubation bottle and $vol_{dilution}$ is the volume sampled for analysis and replaced with fresh culture medium. To estimate the growth-light relationships, we fitted nonlinear models to the observed growth rates using the model of Webb et al. (1974):

$$\mu = \mu_{max}\left(1 - e^{\frac{-\alpha_\mu(PAR - PAR_{comp\mu})}{\mu_{max}}}\right)$$

where μ_{max} is the growth rate at saturating light (d^{-1}), α_μ is the growth efficiency at limiting light ($m^2 E^{-1}$), $PAR_{comp\mu}$ is the compensation light intensity for growth (E m^{-2} d^{-1}) and *PAR* is the daily PAR exposure (E m^{-2} d^{-1}). $PAR_{comp\mu}$, the light intensity when μ = 0, is driven by the balance between photosynthesis at sub-saturating light and the maintenance respiration (Langdon, 1988). We obtained the estimates of the community light acquisition parameters μ_{max}, α_μ and $PAR_{comp\mu}$ (± SE) for the best fitting model. The onset of growth saturation Ik_μ was calculated as follows: $Ik_\mu = (\mu_{max} / \alpha_\mu) + PAR_{comp\mu}$. Light-dependent net oxygen production rates were analyzed the same way. At the daily timescale, P_{max} is the maximal net production rate, α_P is the efficiency of oxygen production at limiting light and Ik_P is the onset of light saturation. At the 20 min timescale, $P_{max,20}$ is the maximal net production rate, $\alpha_{P,20}$ is the efficiency of oxygen production at limiting light and $Ik_{P,20}$ is the onset of light saturation.

To test the effects of effective daylength and nonlinearity on production and growth, we calculated every 75 sec the time proportion spent at irradiances below 10 μE m^{-2} s^{-1} (see Ryther, 1956; Falkowski and Owens, 1978, 1980; Langdon, 1988) and above the onset of light saturation $Ik_{P,20}$.

The efficiency of light-limited ETR (α_{ETR}) and the maximal relative ETR (ETR_{max}) were extracted from the Webb model with null compensation light intensity. The onset of ETR saturation Ik_{ETR} was calculated as follows: $Ik_{ETR} = ETR_{max} / \alpha_{ETR}$.

Differences in photosynthetic parameters between constant and fluctuating light were assessed using non-parametric Kruskal-Wallis test. Parameters differences between incubation depths were assessed with the Kruskal-Wallis post-hoc test using the *kruskalmc()* command from the *pgirmess* R package. Relative photosynthesis was then calculated every 75 sec using incident PAR, α_{ETR} and ETR_{max}, multiplied by the absorption cross-section ($m^2\ mg^{-1}$ Chl *a*) and afterwards integrated by day. The light-dependent daily ETR-based photosynthesis (estimate of the daily gross oxygen production) was fitted with the Webb model.

The linear (growth-dependent night respiration) and nonlinear (light-dependent gross/net production and growth) mixed effects models were implemented with the *nlme* R package (Pinheiro et al., 2018 - library *nlme* R package version 3.1-137) with maximum log likelihood and setting "incubation bottle" as random factor to account for temporal autocorrelation of measurements and ensure independence of errors.

Differences in parameters of the light-dependent gross/net production and growth were assessed using the nonlinear Webb model with "incubation bottle" as random factor. We tested the null hypothesis *i.e.*, that the parameters did not vary between treatments, against the alternative hypothesis that one or more parameters did vary. Conclusions on treatment effect were based on model comparisons with F-tests following Bates and Watts (1988, p. 105ff) and providing p-values. The models selected were also supported by the lowest Akaike information criterion (AIC) (Akaike, 1974; results not shown).

All analyses were performed with R version 3.3.2 (R core team, 2016).

Results

Photosynthesis

Vertically mixed algae had on average significantly higher ETR_{max}, Ik_{ETR} ($p<0.001$), and α_{ETR} ($p<0.01$) than under constant light exposure (Table 1). Under both constant and fluctuating light, all parameters (ETR_{max}, Ik_{ETR} and α_{ETR}) significantly increased with depth ($p<0.001$). Values of α_{ETR} and ETR_{max} did not vary significantly with daily PAR ($p>0.05$).

No parameters of the light-dependent daily ETR-based photosynthesis varied significantly ($p>0.05$) between constant and fluctuating light exposures (Fig. 1, with daily α_{ETR} (relative units) under constant light = 43.95 ± 5.55 and under fluctuating light = 38.37 ± 2.48,

p-fits<0.01). No difference in daily ETR_{max} has been observed, probably because the low number of observations in the saturating range for moved samples.

Table 1. Mean values (± SD) of maximal electron transport rates ETR_{max}, photosynthetic efficiency α_{ETR} (all p-fits<0.001) and onset of electron transport rate saturation Ik_{ETR}. Compensation light intensities for photosynthesis were null. "F" stands for bottles incubated at fixed depths 0, 20, 40 and 80 cm. "L" stands for lifted bottles from the surface to 50, 100 and 180 cm. All depths were aggregated to obtain the mean values (± SD) under constant and fluctuating light.

	ETR_{max}	α_{ETR}	Ik_{ETR}
	(relative units)	(relative units or $(\mu E\ m^{-2}\ s^{-1})^{-1}$)	$(\mu E\ m^{-2}\ s^{-1})$
Constant light	57.8 ± 13.1	0.241 ± 0.022	243.1± 51.5
Fluctuating light	73.1 ± 10.8	0.250 ± 0.014	291.8 ± 40.4
F0	47.3 ± 12.3	0.227 ± 0.022	210.7 ± 39.1
F20	56.3 ± 9.6	0.240 ± 0.016	234.7 ± 37.1
F40	64.6 ± 7.6	0.253 ± 0.011	255.7 ± 33.6
F80	62.6 ± 14.8	0.241 ± 0.027	268.4 ± 70.3
L50	63.8 ± 10.0	0.241 ± 0.015	264.7 ± 35.7
L100	75.4 ± 6.1	0.256 ± 0.010	295.9 ± 31.3
L180	80.6 ± 7.9	0.256 ± 0.012	316.1 ± 36.3

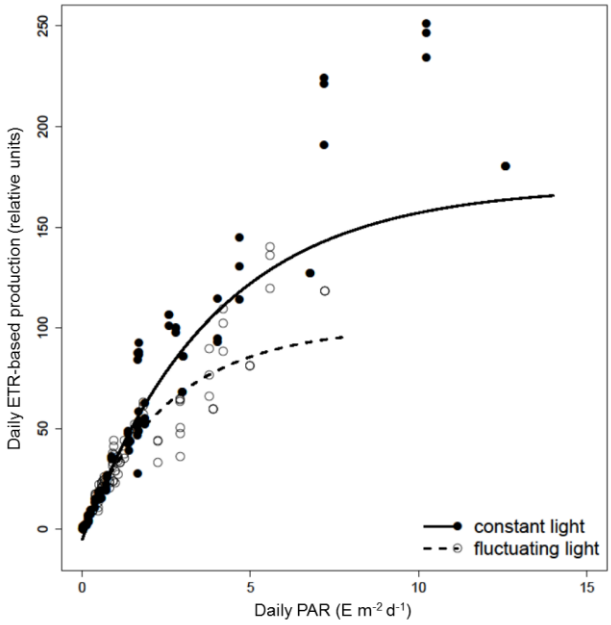


Figure 1. Light-dependent daily ETR-based photosynthesis under constant (full circles, full line) and fluctuating light (empty circles, dashed line). All depths were aggregated for fitting the models under constant (F0, F20, F40 and F80) and fluctuating light (L50, L100 and L180).

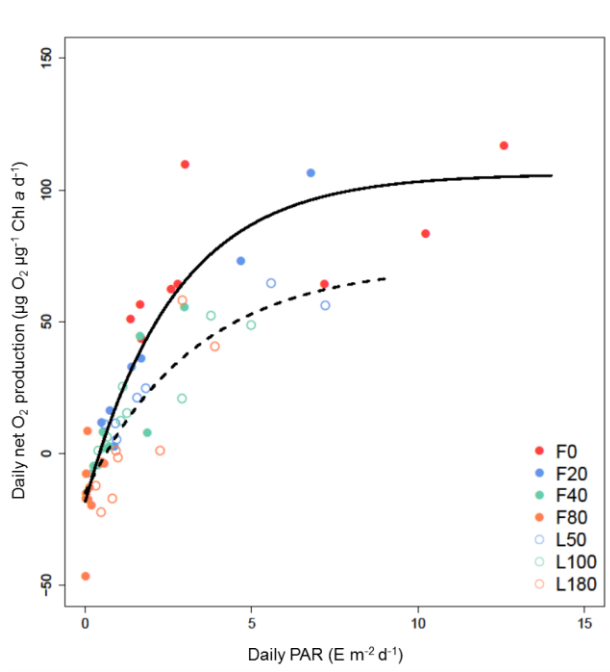


Figure 2. Light-dependency of the community daily net O_2 production rates under constant and fluctuating light. "F" stands for bottles incubated at fixed depths 0, 20, 40 and 80 cm. "L" stands for lifted bottles from the surface to 50, 100 and 180 cm. All depths were aggregated for fitting the models under constant (F0, F20, F40 and F80, full line) and fluctuating light (L50, L100 and L180, dashed line).

Table 2. Extracted parameters (estimate ± SE) from the fits of light-dependent daily net O_2 production rates (all p-fits<0.05). Only α_P was significantly different (p<0.01) between the two light exposures.

	P_{max}	α_P	PAR_{compP}	Ik_p
	($\mu g\ O_2\ d^{-1}\ \mu g^{-1}$ Chl *a*)	($\mu g\ O_2\ \mu g^{-1}$ Chl *a* $E^{-1}\ m^2$)	($E\ m^{-2}\ d^{-1}$)	($E\ m^{-2}\ d^{-1}$)
Constant light	106.13 ± 10.10	39.62 ± 7.99	0.43 ± 0.10	3.11
Fluctuating light	71.89 ± 18.55	21.90 ± 5.31	0.62 ± 0.24	3.90

Daily net oxygen production

The daily net O_2 production rates were higher under constant than under fluctuating light along the daily PAR gradient (Fig. 2). The only significantly higher parameter was the efficiency of net O_2 production α_P (p<0.01, Table 2). The compensation light intensity for daily production was slightly higher under fluctuating light but not significantly different (p>0.05). Finally, the production rate at saturating daily PAR P_{max} was (insignificantly, p>0.05) higher under constant light. Lack of significance of P_{max} could come from both the scattered production rates at saturating daily PAR under fixed incubation and the low number of observations in the saturating range for moved samples. At the specific depth level, only the daily production of surface algae (depth F0) was saturated ($P_{max(F0)}$ = 90.47 ± 12.93 $\mu g\ O_2\ d^{-1}\ \mu g^{-1}$ Chl *a*, p-fit<0.001) and determined the maximal production rate P_{max} of algae experiencing constant light exposure (F0, F20, F40 and F80 aggregated). Similarly, P_{max} of all mixed algae (L50, L100 and L180 aggregated) was based on the production rates of algae mixed from the surface to 50 cm depth ($P_{max(L50)}$ = 70.65 ± 17.31 $\mu g\ O_2\ d^{-1}\ \mu g^{-1}$ Chl *a*, p-fit<0.05). Once again, P_{max} of the mixed algae depended only on very few data points.

Short-term net oxygen production

On September 14th, the average daily PAR at the surface (daily PAR = 1.36 $E\ m^{-2}\ d^{-1}$, Appendix – Table 1) was below the onsets of saturation of daily net O_2 production Ik_P (Table 2). Fitting the light-dependency of the net O_2 production at a 20 min timescale with the Webb model was only possible under constant light (Table 3A). Production rates of surface algae ($P_{max,20(F0)}$ = 2.57 ± 0.76 $\mu g\ O_2\ \mu g^{-1}$ Chl *a* s^{-1}, p-fit<0.01) determined the P_{max} of all fixed algae. The estimated onset of saturation $Ik_{P,20}$ for fixed algae was low (64.1 $\mu E\ m^{-2}\ s^{-1}$). Mean light intensities received by mixing algae were much lower (<30 $\mu E\ m^{-2}\ s^{-1}$) and never saturating.

To compare linear increases in production at sub-saturating light intensities between both light exposures, we fitted linear mixed effects models. The extracted production efficiency under fixed incubation was 1.8 higher than under vertical mixing. Estimated slopes (± SE) were: 0.044 ± 0.0036 ng O_2 μg^{-1} Chl *a* μE^{-1} m^{-2} (p-fit<0.001, similar to the Webb model estimation) at constant light and 0.0254 ± 0.0168 ng O_2 μg^{-1} Chl *a* μE^{-1} m^{-2} (p-fit>0.05 because of the low number of observations) at fluctuating light. $PAR_{compP,20}$ was higher under fluctuating light ($PAR_{compP,20}$ for fluctuating light: 8.82 $\mu E\, m^{-2}\, s^{-1}$, for constant light: 7.23 $\mu E\, m^{-2}\, s^{-1}$). $PAR_{compP,20}$ of fixed algae extracted from the linear model was close to the one estimated by the Webb model (Table 3A). On this very dim day, in comparison with constant depth incubation, vertically mixed algae spent longer parts of the day at irradiances below 10 $\mu E\, m^{-2}\, s^{-1}$ (Fig. 4A). This shortened the effective daylength for mixed algae by 15.6 % compared to fixed algae. Also, mixed algae spent slightly longer parts of the day above $Ik_{P,20}$ (of fixed algae), except for the surface sample (Fig. 4B).

Table 3. Extracted parameters (estimate ± SE) from the light-dependent net O_2 production at a 20 min timescale fitted with the Webb model (all p-fits<0.01 if not stated *ns*) at (A) low daily PAR (September 14th) and (B) high daily PAR (September 9th). The lack of data under fluctuating light on September 14th prevented from fitting the Webb model. No parameters differed significantly (p>0.05) between the two light exposures on September 9th. Parameters between brackets could not be reliably estimated because of the lack of data at saturating light intensities.

		$P_{max,20}$	$\alpha_{P,20}$	$PAR_{compP,20}$	$Ik_{P,20}$
		(ng O_2 μg^{-1} Chl *a* s^{-1})	(ng O_2 μg^{-1} Chl *a* μE^{-1} m^{-2})	($\mu E\, m^{-2}\, s^{-1}$)	($\mu E\, m^{-2}\, s^{-1}$)
(A) September 14th low daily PAR	Constant light	2.81 ± 0.74	0.048 ± 0.018	5.56 ± 2.86 *ns*	64.10
	Fluctuating light	---	---	---	---
(B) September 9th high daily PAR	Constant light	3.40 ± 0.47	0.028 ± 0.006	*Set to 0*	121.43
	Fluctuating light	(8.98 ± 9.01 *ns*)	0.018 ± 0.005	*Set to 0*	(498.89)

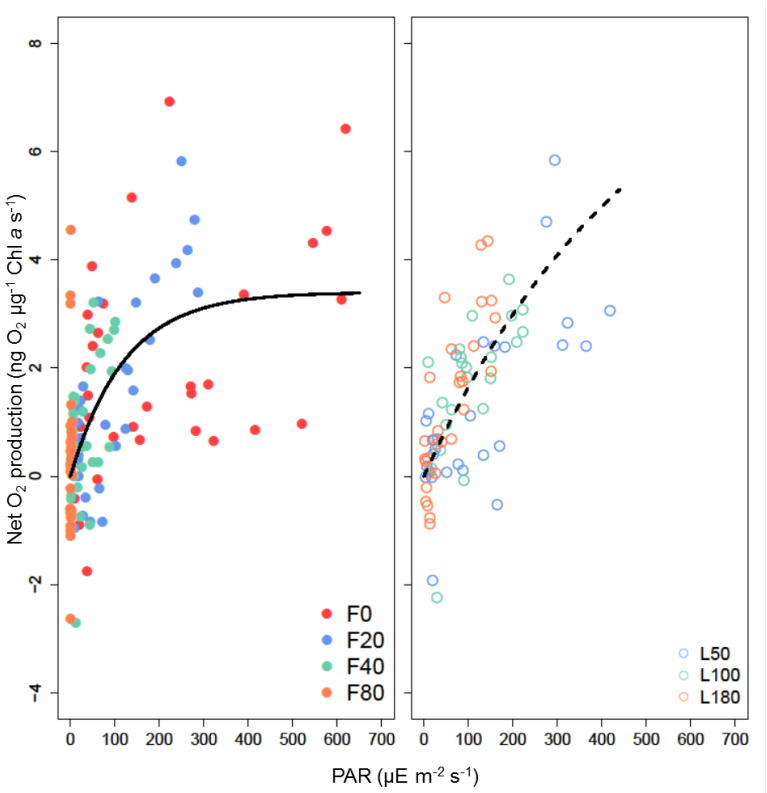


Figure 3. Light-dependency of the net O_2 production at a 20 min timescale under constant and fluctuating light at high daily PAR (September 9th). "F" stands for bottles incubated at fixed depths 0, 20, 40 and 80 cm: "L" stands for lifted bottles from the surface to 50, 100 and 180 cm. All depths per treatment were aggregated for fitting the models under constant (F0, F20, F40 and F80, full line) and fluctuating light (L50, L100 and L180, dashed line).

On September 9th, the average daily PAR at the surface (daily PAR = 10.23 E m^{-2} d^{-1}, Appendix – Table 1) was above the onsets of saturation of daily net O_2 production Ik_P (Fig. 2, Table 2). The 20 min timescale light-dependent production on this day is presented in Figure 3. Only the production of fixed algae was saturated ($Ik_{P,20}$ = 121.43 µE m^{-2} s^{-1}) and no significant differences between parameters of the two light treatments occurred ($p>0.05$, Table 3B). Additionally, we observed higher $P_{max,20}$ (and $Ik_{P,20}$) and lower $\alpha_{P,20}$ on the 9th compared to the 14th of September; but these differences in parameters (extracted from the Webb model) were not significant ($p>0.05$). At the same daily PAR supply, because of the nonlinear production-light relation, vertically mixed algae spent much longer parts of the day at irradiances below 10 µE m^{-2} s^{-1} than fixed algae (Fig. 4A). This shortened the effective daylength for mixed algae by 19.1 % compared to fixed algae. At the same daily PAR, algae incubated at the surface and 20 cm depth were exposed longer to irradiances above $Ik_{P,20}$ than mixed algae (Fig. 4B).

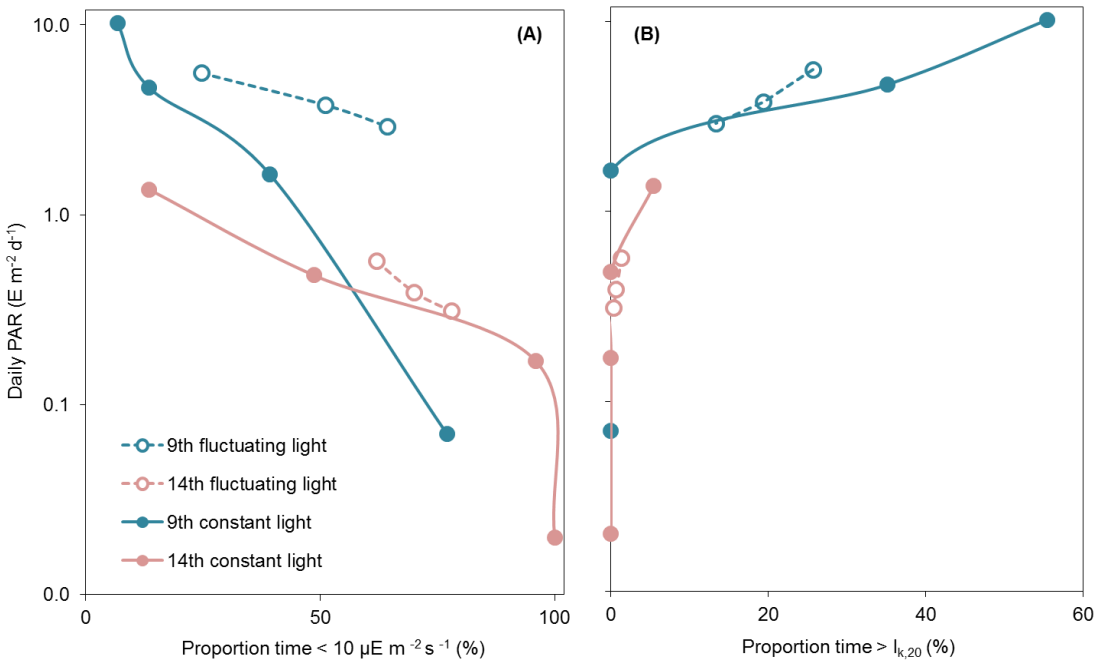


Figure 4. Time proportion spent at irradiances (log scale) (A) below 10 µE $m^{-2}s^{-1}$ and (B) above the onsets of saturation for 20 min production of fixed algae on a sunny and a dim day ($Ik_{P,20}$ for sunny day: 121.43 µE $m^{-2}s^{-1}$, for dim day: 64.10 µE $m^{-2}s^{-1}$). Light attenuation coefficients for the sunny day: 5.22 m^{-1}, for the dim day: 5.19 m^{-1}.

Table 4. Extracted parameters (estimate ± SE) from the depth-specific Webb light-dependent net O_2 production at a 20 min timescale (all p-fits<0.05 if not stated *ns*) on a sunny day (September 9th) at (A) increasing (morning) and (B) decreasing (afternoon) irradiances.

		$P_{max,20}$ (ng O_2 µg^{-1} Chl *a* s^{-1})	$\alpha_{P,20}$ (ng O_2 µg^{-1} Chl *a* µE^{-1} m^{-2})	$PAR_{compP,20}$ (µE m^{-2} s^{-1})	$Ik_{P,20}$ (µE m^{-2} s^{-1})
(A) Increasing irradiances	F0	4.83 ± 0.47	0.079 ± 0.031	2.22 ± 12.54 *ns*	63.36
	F20	6.52 ± 3.83 *ns*	0.026 ± 0.010	*Set to 0*	250.77
	L50	4.16 ± 1.38	0.026 ± 0.016 *ns*	5.31 ± 15.86 *ns*	165.31
(B) Decreasing irradiances	F0	1.25 ± 0.28	0.015 ± 0.006	64.71 ± 17.92	148.04
	F20	---	0.02 ± 0	46.47 ± 19	---
	L50	5.82 ± 24.75 *ns*	0.008 ± 0.006 *ns*	*Set to 0*	727.5

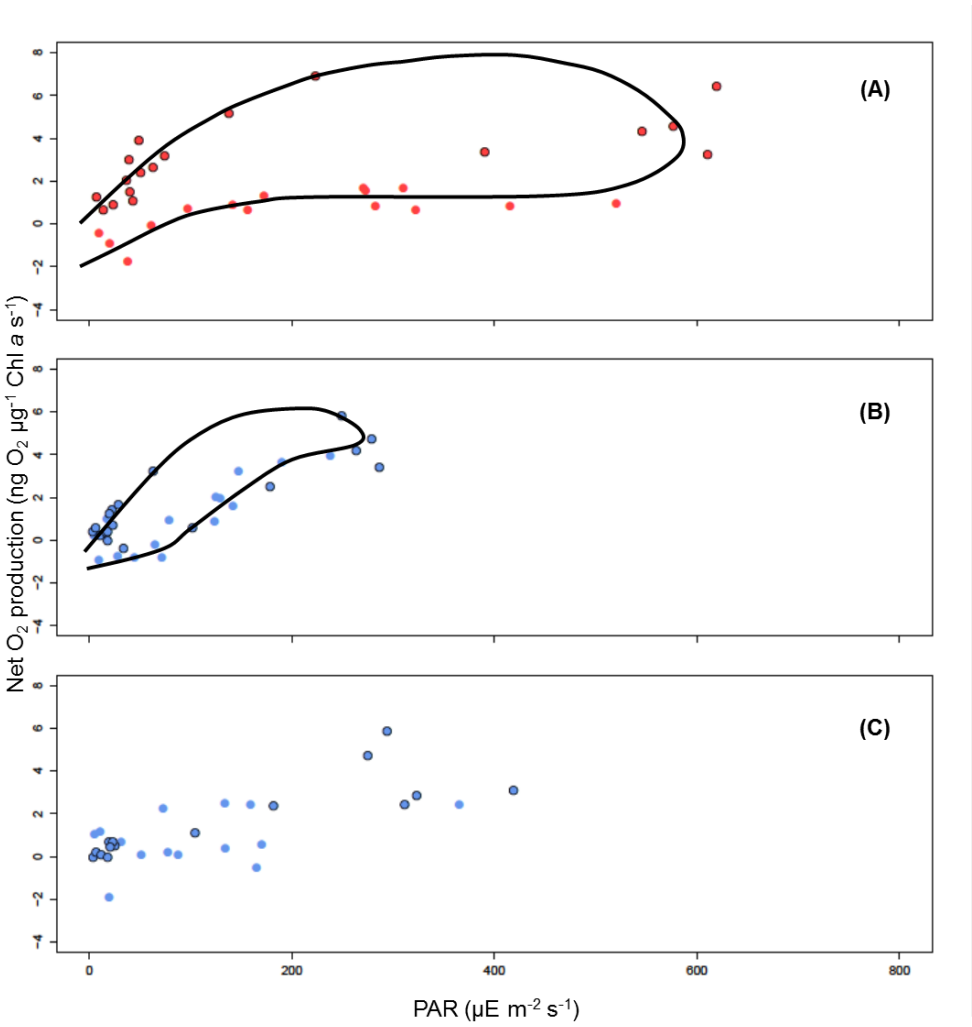


Figure 5. Light-dependency of the net O_2 production at a 20 min timescale at high daily PAR (September 9th) of algae incubated (A) at the surface (B) at 20 cm depth and (C) mixed from 0 to 50 cm depth. Black lines are schematic representations of the production hysteresis occurring between increasing (morning, black rimmed circles) and decreasing (afternoon) irradiances.

The maximal production rate $P_{max,20}$ of fixed algae (F0, F20, F40 and F80 aggregated) at a 20 min timescale was based on the production of surface algae (depth F0). During the sunny day, net O_2 production rates of surface algae increased with light supply (following the diurnal sunlight course, Appendix – Fig. 2) and reached inhibition around 1 pm (maximal irradiance ≈ 600 µE m^{-2} s^{-1}, Fig. 5A). Afterwards, production rates drastically decreased when exposed to the same irradiances in the decreasing order. Because of missing data around $P_{max,20(F0)}$, the Webb model with introduction of a photo-inhibition parameter (Platt et al., 1980) could not have been implemented. Extracted parameters from the Webb model are

given in Table 4. $P_{max,20(F0)}$ and $\alpha_{P,20(F0)}$ significantly decreased ($p<0.001$) between increasing (morning) and decreasing (afternoon) light intensities. This coincided with a significant increase ($p<0.001$) of the compensation light intensity $PAR_{compP,20(F0)}$. Production rates of algae incubated at 20 cm also displayed this hysteresis (Fig. 5B) - but to a lesser extent (differences in $P_{max,20(F20)}$ and $\alpha_{P,20(F20)}$ were not significant, $p>0.05$) as light intensities were about 50 % lower than at the surface (maximal irradiance ≈ 300 µE m^{-2} s^{-1}) and photo-inhibition less pronounced. Only the compensation light intensity $P_{max,20(F20)}$ increased significantly ($p<0.05$) in the afternoon (Table 4). On the contrary, the production of algae mixed from the surface to 50 cm was never saturated/inhibited (Fig. 5C). Yet these algae received similar mean light intensities than the community incubated at 20 cm depth. The parameters $P_{max,20(L50)}$, $\alpha_{P,20(L50)}$ and $PAR_{compP,20(L50)}$ did not differ significantly ($p>0.05$) between increasing and decreasing light intensities (Table 4).

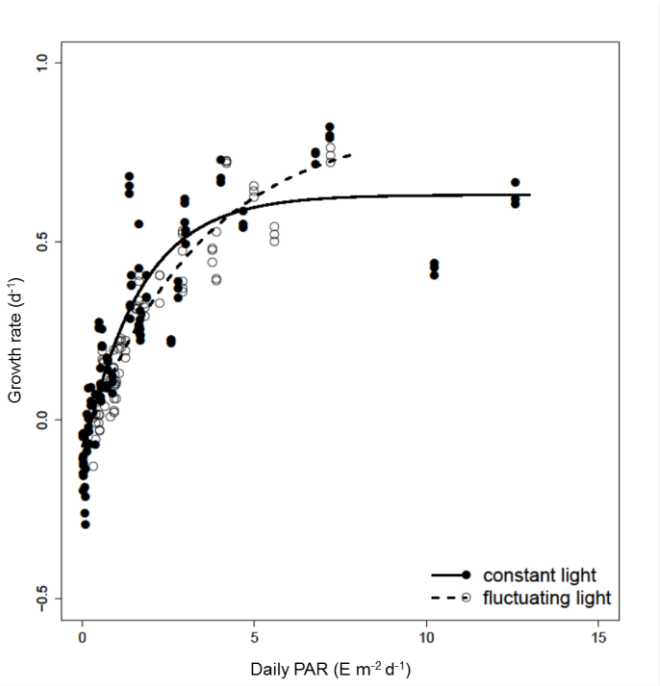


Figure 6. Light-dependent community growth rates under constant (full circles, full line) and fluctuating light (empty circles, dashed line). All depths were aggregated for fitting the models under constant (F0, F20, F40 and F80) and fluctuating light (L50, L100 and L180).

Night respiration and growth

Chl *a*-specific night respiration rates increased linearly with growth only at fixed incubation depths (night respiration = 1.26 * growth + 1.22, R^2 = 0.25 and p-fit<0.05). In contrast, night respiration rates of mixing algae did not depend on growth rates ($p>0.05$).

The light-dependent community growth rates are presented in figure 6. The community growth efficiency α_μ was significantly higher under constant than under fluctuating light ($p<0.01$, Table 5). The compensation light intensities $PAR_{comp\mu}$ for growth were similar ($p>0.05$). The maximal community growth rate μ_{max} was higher under fluctuating than under constant light albeit not significantly different ($p>0.05$). Finally, the onset of growth saturation Ik_μ was two times higher under fluctuating compared to constant light supply.

Table 5. Extracted parameters (estimate ± SE) from the fits of the light-dependent growth rates (all p-fits <0.01). Only α_μ was significantly different ($p<0.01$) between the two light exposures.

	μ_{max}	α_μ	$PAR_{comp\mu}$	Ik_μ
	(d^{-1})	($m^2 E^{-1}$)	($E m^{-2} d^{-1}$)	($E m^{-2} d^{-1}$)
Constant light	0.63 ± 0.03	0.39 ± 0.04	0.26 ± 0.04	1.88
Fluctuating light	0.84 ± 0.05	0.24 ± 0.02	0.29 ± 0.07	3.79

Discussion

At sub-saturating light

At sub-saturating light, the photosynthetic efficiency α_{ETR} was enhanced under vertical mixing. Despite this, the efficiency of ETR-based daily photosynthesis calculated every 75 sec did not differ between both light regimes. More, efficiencies of daily production α_P and growth α_μ were significantly higher under constant than under fluctuating light. This apparent contradiction was also observed in a similar experiment under semi-natural conditions in the Chinese Three Gorges Reservoir (Köhler et al., 2018). Obviously, the response of phytoplankton to fluctuating light depends on the studied time-scale. In the short-term (seconds to minutes), light fluctuations seem to enhance photosynthesis. On a daily scale, mixing algae experience periods of very low light that shorten the effective daylength, reduce the efficiency of light utilization and trigger acclimation processes. In the following, we discuss the relative importance of these mechanisms.

Only periods of the day with light intensities above 10 µE m^{-2} s^{-1} (see Ryther, 1956; Falkowski and Owens, 1978, 1980; Langdon, 1988) are considered as available for net production ("effective daylength"). This threshold is similar to compensation light intensities

extracted from the 20 min linear net production-light relations on September 14th ($PAR_{compP,20}$ for fluctuating light: 8.82 µE m^{-2} s^{-1}, for constant light: 7.23 µE m^{-2} s^{-1}). At the same daily PAR (and on both days) mixed algae were longer exposed to light intensities below 10 µE m^{-2} s^{-1} compared to fixed algae. This shortened the effective daylength for mixed algae by 19.1 % on September 9th and 15.6 % on September 14th compared to fixed algae and *de facto*, explains part of the decline in the efficiency of daily production α_P and growth α_μ at sub-saturating light intensities. Reduced effective daylength induced by light fluctuations has been proven to decrease species-specific growth rates of algae incubated under fluctuating light compared to under constant light at the same daily PAR (Nicklisch, 1998; Nicklisch and Fietz, 2001; Nicklisch et al., 2008; Shatwell et al., 2012). More, lowering the z_{eu}:z_{mix} ratio (or shorten the effective daylength) under nutrient-replete conditions in the laboratory led to lower α_μ of the diatom *Stephanodiscus neostraea* and the cyanobacterium *Planktothrix agardhii* (Nicklisch and Fietz, 2001).

Nonlinearity of the light-dependency of production and growth was also hypothesized to be responsible for the decline in α_P and α_μ at vertical mixing (Thornley, 1974; Dromgoole, 1988; Litchman, 2000). Indeed, saturating light intensities allow for less production or growth per available photon than under sub-saturating light. Thus, α should be lower when the light supply fluctuates around Ik than when the light supply is constant and sub-saturating at the same mean intensity. In the present study we used the $Ik_{P,20}$ from fixed algae and applied it for both treatments. We used $Ik_{P,20}$ from fixed and not mixed algae for three reasons: 1/ this parameter at vertical mixing would remain the average of a broad range of light intensities for mixed algae 2/ for a better comparability of treatments and 3/ $Ik_{P,20}$ is defined for constant light exposure and this requirement was fulfilled only for stationary and not for vertically moved samples. During the dim day, mixed algae spent less than 2 % of daytime above $Ik_{P,20}$. At fixed incubation depths, only surface algae were exposed to irradiances above the onset of saturation (5.4 % of daytime). During the sunny day, mixed algae spent on average 19.5 ± 6.2 % of daytime at irradiances above $Ik_{P,20}$ whereas all fixed samples incubated below 40 cm depth received only limiting light. Only algae incubated at the surface and 20 cm depth spent longer periods at irradiances above $Ik_{P,20}$ (55.4 % at the surface and 35.1 % of daytime at 20 cm depth) than mixed algae. Overall, most samples received limiting light even during most of the sunny day. Thus, it seems unlikely that nonlinearity of light-dependent production played a prominent role in the decline of α_P and α_μ under fluctuating light exposure.

The daily ETR was modeled using incident light and the parameters of the ETR-light relation measured for dark-adapted algae. Thus, they cover consequences of different frequency distribution of underwater light (effective photoperiod and nonlinearity) on the production-light relation but exclude effects of diurnal light acclimations or light-dependent losses. In our experiment, mixing caused declines of 39 % in growth rate and 13 % in daily ETR. In a similar experiment, Köhler et al. (2018) found a reduction in growth rate of 64% and in daily ETR of 47%. Thus, a large part of the observed decline in α_P and ultimately α_μ under fluctuating compared to constant light can be directly explained by the different frequency distributions of underwater light. This estimation can be modeled if light distributions and ETR-light parameters are known and may be further improved by taking into account the diurnal variations in electron transport rates. The remaining difference should be attributed to diurnal changes (including acclimation processes) in photosynthesis and losses for biosynthesis.

Enhanced losses for biosynthesis (*e.g.* respiration when algae experience effective darkness) and/or costs of acclimation of mixed algae to fluctuating light may have also contributed in reducing growth rates. Measurements of algae respiration in semi-natural conditions remain challenging because of the simultaneous oxygen consumption by bacteria and very small grazers. No difference in night respiration rates after sub-saturating light was noted between the two incubation types. Nonetheless, some studies observed higher rates of respiration (Grande et al., 1989; Luz et al., 2002) and exudation (Zlotnik and Dubinsky, 1989; Marañón et al., 2004) in the light compared to the dark. It is thus likely that the net oxygen production has been influenced by other losses such as exudation or day respiration (Cosper, 1982), which were not quantified in the present study. The acclimatization costs of algae to fluctuating light are very complex to determine because the nonlinear light-dependency of production and growth implies that they vary according to the mean irradiance and also to the temporal light distribution (Litchman, 2000). The ability to cope with light fluctuations is species-dependent since they differ in many aspects of their cellular components, physiology, evolutionary history and acclimatization potential (Glover et al., 1987; Litchman, 2000; Gregory, 2001; Yoon et al., 2004; Shatwell et al., 2012; Guislain et al., 2019). In addition, acclimation to light fluctuations is timescale-dependent (Falkowski, 1984; MacIntyre et al., 2000). Some acclimatization strategies to constant light are well-understood. For instance, algae acclimated to constant low light are expected to allocate more energy into light-harvesting machinery (Geider et al., 1996), thus the chlorophyll : C

ratio or the content of accessory pigments should increase. In contrast, even at on average daily limiting light, mixing algae may have to face intermittent exposure to limiting, saturating and even inhibiting irradiance. Inevitably, the mechanisms that protect against strong light impair the efficiency of photosynthesis and growth at low light (MacIntyre et al., 2002; Talmy et al., 2013). As a result, mixing algae should invest fewer resources into light-harvesting machinery at low mean light intensities compared to stationary algae while investing into photo-protective mechanisms such as energy dissipating pigments of the xanthophyll cycle (Cullen and Lewis, 1988; Geider et al., 1996; Havelková-Doušová et al., 2004; Talmy et al., 2013). These acclimation processes to fluctuating light supply of on average limiting intensity decrease eventually the energy allocated to growth.

Our experimental setup provides only a coarse approximation to complex mixing conditions. In natural environments, the movement of phytoplankton through the water column is certainly less predictable than applied in our experiment (Macintyre, 1993). Thus, more irregular movement of algae in the lake may require more flexibility and perhaps faster responses to light fluctuations that may even more hamper the conversion efficiency of energy into biomass.

At saturating light

Maximal daily ETR-based photosynthesis and net oxygen production P_{max} were slightly higher under constant than under fluctuating light supply ($p>0.05$). Nevertheless, the maximal growth μ_{max} of mixed algae was slightly higher than of algae incubated at fixed depths, albeit non-significantly different. Similar μ_{max} between constant and fluctuating light exposures have been already observed in the laboratory (Litchman, 2000; Dimier et al., 2009) but also in semi-natural conditions (Köhler et al., 2018). Theoretically, similar μ_{max} at constant and fluctuating light can be expected if most of the fluctuating light exceeds Ik_{μ} and is not inhibiting, *i.e.* at low optical depths during sunny days. The onset of growth saturation Ik_{μ} was twice as high under fluctuating than under constant light (Table 5), is meaningful only at the 24 h timescale and already integrates diurnal changes in photosynthesis and light-dependent losses (Gibson and Foy, 1983). The analysis of the short-term net production countered this problem. During the whole sunny day (September 9th), only the samples incubated at the surface and at 20 cm depth were exposed longer than mixing algae to irradiances above $Ik_{P,20}$ (Fig. 4B). We observed that the short-term net production (oxygen consumption by exudation and respiration included) of F0 and F20 algae exposed to

increasing irradiances in the morning was higher than when exposed to the same irradiances in the afternoon in the decreasing order. This hysteresis is explained by diurnal changes in oxygen production and/or consumption. Photo-inhibition of surface and to a lesser extent near water surface algae by PAR (and UV-A, see Cullen at al., 1992) could explain the decline in net oxygen production rates and ultimately energy allocated to growth for surface algae. Impact of photo-inhibition on production is two-fold. First, inhibiting irradiances increase the metabolic costs for photo-protection and repair of the photosystems. Excessive photosynthetic excitation may damage the photosystems and result in additional metabolic requirements such as for protein turnover (*e.g.* for protein D1 of the Photosystem II, Richardson et al. 1983; Geider and Osborne, 1989; Ferris and Christian, 1991; Long et al., 1994; Raven, 2011). Second, photosystems activity is inevitably impaired during photorepair, leading to photosynthesis foregone (Raven, 2011). The significant reduction of ETR_{max} and Ik_{ETR} of surface algae compared to deeper algae even after 12 h darkness demonstrated substantial long-term effects of photo-inhibition. Köhler et al. (2018) also found increasing ETR_{max} and Ik_{ETR} with depth at the Three Gorges Reservoir (China). In the present study, we observed a significant increase of the compensation light intensity for short-term production of surface ($PAR_{compP,20(F0)}$) and near surface algae ($PAR_{compP,20(F20)}$) in the afternoon. Thus, we may speculate that photo-inhibition (at irradiances > $Ik_{P,20}$) reduced the production efficiency $\alpha_{P,20}$ and/or increased additional respiratory maintenance costs (increasing $PAR_{compP,20}$) at very low irradiances (below < $Ik_{P,20}$) (Langdon, 1988; Geider and Osborne, 1989). The hysteresis we observed could be also due to a more intense carbohydrate synthesis and utilization for protein synthesis with increasing irradiance. Consequently, dark respiration would increase with light intensity and thus explain the increase in compensation light intensity (Falkowski and Owens, 1978). Also, only night respiration rates of fixed algae increased significantly with growth to fuel biosynthesis and maintenance (Geider and Osborne, 1989). Therefore, from our results, it is clear that day and night respiration plays a major role in balancing the additional production observed at constant compared to fluctuating light.

Mixed algae attained slightly higher μ_{max} while producing less ($p>0.05$) than fixed algae at saturating light. This may indicate lower losses of mixing algae. Night respiration rates increased with growth rates under constant, but not under fluctuating light. Perhaps, mixing algae covered their increasing energy demand for biosynthesis and maintenance at faster growth by dark respiration during the day. Unfortunately, algal respiration in the light

remains highly challenging to measure under the experimental conditions applied and was not quantifiable from the net oxygen production measurements. Yet, it is very likely that mixed algae took advantage of cyclic periods in effective darkness for rapidly triggering respiration, relaxing their photosystems and efficiently coupling light and dark reactions during the day, especially in highly turbid systems such as Lake TaiHu (Beardall et al., 1994; Ibelings et al., 1994; Helbling et al., 2013). It is also possible that the 20 min net oxygen production integrated any rapid changes in photorespiration and other reactive oxygen species scavengers (such as the Mehler rection, *e.g.* Wagner et al., 2006) when algae circulated through the vertical light gradient. In contrast to surface algae, we observed no hysteresis in production nor increased compensation light intensity of mixed samples throughout the sunny day. Vertically mixed algae were not exposed long enough to surface irradiance to inhibit their net production. In fact, the effects of strong light exposure on algal photosynthesis and growth are dosage-dependent and the mitigation of inhibition by turbulent mixing has been already demonstrated (Marra, 1978; Grobbelaar, 1985; Ibelings et al., 1994; Neale et al., 1998; Köhler et al., 2001). This avoidance of inhibition by intermittent exposure to high light intensities depends, among other factors, on the ratio between mixing and euphotic depths (Köhler et al., 2001). Vertically mixed algae have been shown to develop efficient strategies in order to avoid inhibition and better exploit light peaks while briefly exposed to the surface irradiance (Ferris and Christian, 1991 for review). In the present study, the average maximal photosynthesis (ETR_{max}) of all mixed algae was significantly higher than of fixed algae. This enhancement of potential photosynthesis under fluctuating light supply has been already reported in laboratory conditions for monocultures (Shatwell et al., 2012) but also under semi-natural conditions for lake communities (Köhler et al., 2018). It allows mixed algae to take advantage of intermittent high light peaks when briefly exposed at the surface while avoiding photo-inhibition (Kana and Glibert, 1987). In comparison with fixed algae, coupled photosynthesis enhancement and respiration of mixed algae would stabilize their respiration to production ratio over a range of mixing depths (constant $PAR_{compP,20}$). Overall, at vertical mixing, algae may accumulate carbohydrates during light peaks and mobilize these resources (respiratory pathway involved) during intermittent exposure to efficient darkness for maintenance and biosynthesis (Geider and Osborne, 1989).

Conclusion and outlook

The present study sheds some light on the light-dependent growth, gross/net oxygen production and respiration of algae communities experiencing vertical mixing. Intermittent exposure to low and high light intensities forced mixed algae to develop alternative light strategy. Compared to fixed algae, they harvest and exploit surface irradiance more effectively by enhancing potential photosynthesis while avoiding photo-inhibition and likely rapidly enhancing respiration when located in effective darkness. Nevertheless, this came with the cost of lower efficiency at low light. The efficiencies of growth and daily production declined under fluctuating light of on average sub-saturating intensity (compared to constant light exposure) mostly because of shortened effective daylength and light acclimation strategies (probable higher respiration rates in effective darkness and photo-protection to intermittent surface irradiance) of mixed algae to fluctuating irradiances.

Our observations indicated that analyzing the light-dependent production/growth/night respiration together allows for a better understanding of the eco-physiology of phytoplankton experiencing vertical mixing. This should improve our predictions of phytoplankton development at vertical mixing. We expect this work to motivate further investigations on light-dependent losses such as dark respiration under fluctuating light.

Acknowledgements - We acknowledge Wenhao Ding for logistic help during our experiment and Jason Galloway for proofreading the manuscript. Facilities were provided by the Nanjing Institute of Geography and Limnology and Chinese Academy of Sciences. We thank Pr. Qin for offering these facilities.

Funding – This work is part of the Sino-German water supply project SIGN funded by the German Ministry of Education and Research (Grant number 02WCL1336D).

Author Contributions Statement

AG lead writer, designed and performed the experiment, analyzed and interpreted the results. JK designed and performed the experiment and commented on the manuscript.

Conflict of Interest Statement

None declared.

Chapter 3

Manuscript title:

How does the cyanobacterium *Microcystis aeruginosa* respond to fluctuating light? A minute-based analysis of photosynthesis and respiration.

Alexis Guislain[1,2*] and Jan Köhler[1]

[1]Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Department of Ecosystem Research, Berlin, Germany.

[2]University of Potsdam, Department of Ecology and Ecosystem Modeling, Potsdam, Germany.

Manuscript in preparation.

Abstract

Phytoplankton receive light of fluctuating intensity during transport in mixing water columns. Their photosynthesis and respiration depend on previous and current light intensities. These physiological processes are tightly connected, but their interplay at rapid light fluctuations is not well understood. We hypothesize that mixing phytoplankton enhance their maximum photosynthesis near the water surface and relax their photosystems and increase respiration in darker layers. In turbid systems, light supply probably fluctuates faster than phytoplankton can acclimate; we therefore expect some hysteresis.

To test these hypotheses, we studied electron transport rates and net oxygen production of a toxic strain of *Microcystis aeruginosa* (FACBH 1322) incubated under fluctuating light (20 min period) throughout the day (12:12 photoperiod) at a single minute timescale. Data were compared with incubations under constant light of the same daily intensity. We observed for the first time hysteresis in oxygen net production of cyanobacteria between increasing and decreasing irradiances within single light cycles. Electron transport rates, however, did not differ between increasing and decreasing light of the same intensity. Therefore, the hysteresis in net oxygen production should be caused by a light-dependent increase of respiration at decreasing light intensities at a time-scale of seconds to minutes. Respiration at decreasing irradiance was positively related to the maximal production rates at prior increasing irradiance. Under fluctuating light exposure, *Microcystis* seemed to accumulate photosynthetic products at high light intensities and mobilized these fresh resources by rapidly enhancing dark respiration for maintenance and biosynthesis. Contrary to the common perception, respiration seems to be very dynamic. Such rapid acclimations to fluctuating light are crucial for the fate of planktonic populations in mixing waters.

Keywords: Fluctuating light, Photosynthesis, Oxygen production, Respiration, Hysteresis, Tradeoff

Introduction

As the major primary producers on Earth, phytoplankton are responsible for about half of the global net production of photosynthetic organisms (Field et al., 1998) and therefore greatly affect food webs and biogeochemical cycles (Falkowski et al., 1998; Litchman et al., 2015). Photosynthetically active radiation (PAR) controls algal growth directly but also influences both the temperature (Edwards et al., 2016) and the nutrient dependency of growth (Litchman et al., 2004b). Light is a highly dynamic resource (Falkowski, 1984; Ferris and Christian, 1991a). In nature, global radiation fluctuates according to seasons, diurnal changes as well as cloud cover. In the water column, light decreases exponentially with increasing optical depth (product of depth and vertical light attenuation). Superimposed, suspended algae experience light of fluctuating intensity during transport in the mixing layer (Kirk, 1994). Vertically mixed phytoplankton have been shown to develop efficient strategies to better exploit surface light peaks while avoiding photo-inhibition (Ferris and Christian, 1991; Iluz et al., 2012; Abu-Ghosh et al., 2015). In order to do so, these organisms usually enhance their photosynthesis and may invest a significant amount of energy into protection against strong irradiance (Marra, 1978; Kana and Glibert, 1987; Dubinsky and Stambler, 2009; Shatwell et al., 2012; Talmy et al., 2013; Köhler et al., 2018). Several authors argued that mixing phytoplankton take advantage of cyclic periods in effective darkness for rapidly triggering respiration, relaxing their photosystems and efficiently coupling light and dark reactions during the day (Beardall et al., 1994; Ibelings et al., 1994; Helbling et al., 2013). But, dark respiration may also be enhanced in the light (Beardall et al., 1994; Hotchkiss and Hall, 2014). As a matter of fact, at vertical mixing, phytoplankton should accumulate carbohydrates during light peaks and mobilize these resources by rapidly triggering respiratory pathways for metabolic maintenance and biosynthesis (Geider and Osborne, 1989). So far, rapid coupled changes in photosynthesis and respiration of eukaryotic and prokaryotic phytoplankton under fluctuating light supply remain largely unknown. Yet, these conjugated acclimations are of major importance for estuaries and shallow wind-exposed lakes ecology for which fast changes from darkness to bright light are typical.

The present laboratory study compares the electron transport rate and net oxygen production of a toxic strain of *Microcystis aeruginosa* (FACBH 1322, cyanobacterium) incubated under fluctuating and under constant light supply. The fluctuating light treatment (20 min period light cycle) replicated to some extent the full overturn of typical Langmuir cells

in mixed lake water layers (Denman and Gargett, 1983; Schubert and Forster, 1997; Thorpe, 2004). Measurements were made on a single minute timescale. In this way, we intend to indirectly detect any rapid changes in respiration occurring within 20 min cyclic changes in light supply. We hypothesized:

H1. A photosynthesis enhancement under fluctuating light compared to constant light exposure.

H2. A rapidly triggered light-enhancement of respiration within a 20 min light cycle that affects negatively net oxygen production of *Microcystis* and increases the compensation light intensity for production.

H3. That higher photosynthesis at increasing light supply leads to higher respiration at the minute timescale.

Material and methods

Experimental conditions

We investigated the freshwater phytoplankton species *Microcystis aeruginosa* (cyanobacterium, toxic strain FACBH 1322). For convenience we will refer to this species by its genus name. It was isolated from Lake TaiHu by the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China. *Microcystis aeruginosa* is highly relevant for Lake TaiHu ecology. Indeed, Lake TaiHu (China, 31°14'N, 120°8'E) is a hypertrophic, wind-exposed, shallow and well-mixed lake that suffers from very intense (up to reach 1000 km^2) and frequent (from May to November, see Duan et al., 2009) cyanobacterial blooms (mostly *Microcystis* spp.). *Microcystis* was grown in semi-continuous culture at 20°C in a fully synthetic freshwater medium MIII-KS (Nicklisch et al., 2008 for detailed composition). Monoculture was maintained in 500mL Erlenmeyer flasks with 400mL suspension volume. Flasks were sealed with an inverted glass beaker and placed on an orbital shaker at 65 revolutions per minute.

The population was cultured in triplicates in two different light conditions (12:12 photoperiod) inside separate climate chambers. The so-called constant light treatment refers to sinusoidal shaped light supply similar to the diurnal course of sunlight. The so-called fluctuating light treatment refers to sinusoidal shaped light supply superimposed with 20 min

light fluctuations with amplitude ranging from I_{max} to 1% I_{max} (Appendix – Figure 1). The fluctuating light treatment replicated to some extent the full overturn of typical Langmuir cells in mixed lake water layers (Denman and Gargett, 1983; Schubert and Forster, 1997; Thorpe, 2004). Mean light exposures per 20min were similar between constant and fluctuating light (Appendix – Figure 2). Daily photosynthetically active radiation doses (daily PAR expressed in E m^{-2} d^{-1}) were similar between treatments (constant light: 6.58; fluctuating light: 6.53).

Light was provided by computer-controlled (LabView program (National Instruments, USA) via DMX/PWM 16-bit, 4-channel controller from LTECH (China)) LEDs (LEDlight flex 15 RGBA and 07 HP, Barthelme, Germany). We measured light intensity in the chambers with a spherical PAR sensor (QSPL2101, Biospherical Instruments, USA).

Microcystis was acclimated to their experimental light conditions for 11 days prior measurements. To ensure similar total biomass between culture flasks, subsamples were taken every morning and replaced with MIII-KS fresh culture medium. The flasks were re-incubated in the climate chambers within 10 min. At the 12th day of acclimation, subsamples were taken without fresh medium refill under sterile conditions at the beginning of the photoperiod (t0), 6 hours (t6) and 12 hours later at the end of the photoperiod (t12).

Measurements

After filtration on Whatman GF/F filters, biomass (µg Chl *a* L^{-1}, Appendix - Table 1) was measured at t0, t6 and t12 by HPLC. This method is detailed in Shatwell et al., 2012).

After at least 30 minutes dark adaptation in opaque bottles, photosynthesis-light curves of *Microcystis* incubated under constant and fluctuating light were measured in triplicates with the PHYTO-PAM (Walz, Germany) at t0, t6 and t12. After 60 seconds acclimation to each light step, Chl *a*-specific relative electron transport rates (ETR) were measured at 10 increasing light intensities (1-1062 µE m^{-2} s^{-1}) and then at the same 10 light steps in the decreasing order, so that a whole cycle lasted 20min.

Oxygen concentrations were recorded continuously (15 seconds time step) in duplicated 45mL flatbed flasks using sealed optodes (DP-PSt3, PreSens, Germany) and a 10 channels fiber optic oxygen transmitter (OXY-10, PreSens, Germany). Prior measurement, samples were bubbled with N_2 to reach about 15% air saturation. Measured oxygen concentrations were normalized during the day by the mean biomass measured in the morning (t0) and afternoon (t6). Only *Microcystis* incubated under fluctuating light

experience 20min light cycles. For comparison with the 20min ETR, we present solely the net oxygen production of *Microcystis* incubated under fluctuating irradiance. Flasks remained for the day within the fluctuating light chamber. Net production rates were calculated from minute changes in mean oxygen concentrations (averaged for a single minute) and related to the mean PAR (µE m^{-2} s^{-1}) received by the cyanobacterium during this period.

Calculations and statistics

The ETR-light relationships were fitted using the Platt model (Platt et al., 1981):

$$\mathrm{ETR} = \mathrm{ETR_S} * \left(1 - \mathrm{e}^{\frac{-\alpha_{\mathrm{ETR}} * \mathrm{PAR}}{\mathrm{ETR_S}}}\right) * \mathrm{e}^{\frac{-\beta_{\mathrm{ETR}} * \mathrm{PAR}}{\mathrm{ETR_S}}}$$

with α_{ETR} the initial slope of the relationship, β_{ETR} the photo-inhibition parameter, *PAR* the light intensity and ETR_S the parameter whose relationship with the maximal electron transport rate ETR_{max} being:

$$\mathrm{ETR_{max}} = \mathrm{ETR_S} * \left(\frac{\alpha_{\mathrm{ETR}}}{\alpha_{\mathrm{ETR}} + \beta_{\mathrm{ETR}}}\right) * \left(\frac{\beta_{\mathrm{ETR}}}{\alpha_{\mathrm{ETR}} + \beta_{\mathrm{ETR}}}\right)^{\frac{\beta_{\mathrm{ETR}}}{\alpha_{\mathrm{ETR}}}}$$

Compensation light intensity for electron transport rate was set to zero.

Separate ETR-light relationships were implemented with the *nls* R package at increasing (1-1062 µE m^{-2} s^{-1}) and decreasing (946-14 µE m^{-2} s^{-1}) irradiances. We obtained the estimates of the photosynthetic traits ETR_{max} and α_{ETR} (± SE) for the best fitting model. The effects of irradiance direction (increasing *vs.* decreasing) and light regime (constant *vs.* fluctuating) on the photosynthetic traits were tested using the non-parametric Wilcoxon test. Time effect was tested using the Kruskal-Wallis test and its multiple comparison test (between t0, t6 and t12) using the R command *kruskalmc()*.

The net oxygen production-light relationships were also fitted using the Platt model (Platt et al., 1981). With P_{max} the maximal net oxygen production rate (ng 0_2 μg^{-1} Chl *a* min^{-1}), α_P the production efficiency (rel. unit), PAR_{compP} the compensation light intensity for

production (µE m^{-2} s^{-1}) and *PAR* the light intensity (µE m^{-2} s^{-1}). We obtained the estimates of the production traits P_{max}, α_P and PAR_{compP} (± SE) for the best fitting model.

For all 20 min light cycles, separate production-light relationships of *Microcystis* incubated under fluctuating light were implemented with the *nls* R package at increasing and decreasing irradiances.

All analyses were performed with R ver. 3.3.2 (<www.r.project.org>).

Results

Electron transport rate

ETR-light relationships were fitted using the Platt model because of a small decline of ETR at strong light intensities (Appendix-Figure 3). In contrast to *Microcystis* incubated under constant light, ETR_{max} values of *Microcystis* incubated under fluctuating light significantly increased during the photoperiod (Table 1, $p<0.05$). Maximal electron transport rates of *Microcystis* experiencing fluctuating light were significantly higher ($p<0.05$) at t12 than at t0. ETR_{max} was always higher ($p<0.05$ at t0, 6 and $p<0.01$ at t12) for *Microcystis* incubated under fluctuating than under constant light. However, under both light treatments, values of α_{ETR} did not vary during the photoperiod ($p>0.05$) and α_{ETR} was always similar ($p>0.05$) between constant and fluctuating light.

Table 1. Replicates averaged (±SD) values of ETR_{max} and α_{ETR} under constant (top) and fluctuating light (bottom). Platt models were fitted on ETR measured at the beginning (t0), middle (t6) and end of the photoperiod (t12) with no regards to the order of irradiance levels. Compensation light intensity for ETR was set to zero.

	ETR_{max}		α_{ETR}	
Constant light	average	SD	average	SD
t0	65.70	2.10	0.24	0.01
t6	68.38	1.56	0.25	0.01
t12	67.41	1.43	0.24	0.01
Fluctuating light				
t0	69.45	2.51	0.24	0.01
t6	71.70	1.93	0.25	0.00
t12	72.91	0.87	0.25	0.01

As shown on Table 2, we observed no significant ($p>0.05$) differences in ETR_{max} and α_{ETR} between increasing (1 to 1062 µE m^{-2} s^{-1}) and decreasing irradiance (946 to 14 µE m^{-2} s^{-1}). This was true for both light treatments at the beginning (t0), middle (t6) and at the end of the photoperiod (t12).

Table 2. Replicates averaged (±SD) values of ETR_{max} and α_{ETR} under constant (top) and fluctuating light (bottom). Separated Platt models were fitted on ETR measured at increasing (1 to 1062 µE m^{-2} s^{-1}) and decreasing irradiance I* (946 to 14 µE m^{-2} s^{-1}) at the beginning (t0), middle (t6) and end of the photoperiod (t12). Compensation light intensity for ETR was set to zero.

		ETR_{max}		α_{ETR}	
Constant light		average	SD	average	SD
t0	increasing I*	65.62	1.54	0.24	0.00
	decreasing I*	65.79	2.94	0.24	0.00
t6	increasing I*	68.57	1.96	0.25	0.01
	decreasing I*	68.19	1.47	0.25	0.00
t12	increasing I*	66.57	1.36	0.25	0.00
	decreasing I*	68.24	1.07	0.24	0.01
Fluctuating light					
t0	increasing I*	68.35	2.17	0.25	0.01
	decreasing I*	70.55	2.73	0.24	0.01
t6	increasing I*	70.49	2.19	0.25	0.00
	decreasing I*	72.91	0.30	0.25	0.00
t12	increasing I*	73.17	0.17	0.24	0.00
	decreasing I*	72.66	1.29	0.25	0.01

Net oxygen production rate

Within all 20min light cycles, net production rates of *Microcystis* exposed to increasing irradiance levels were higher than when exposed to the same intensity but in the decreasing order (Fig. 1 for example). This so-called hysteresis in net production rates impacted the values of the extracted production parameters at increasing and decreasing irradiance. After 8 hours of incubation, production rates became very scattered and those measured at decreasing irradiance levels were always negative. Therefore, only the 8 first hours of incubation were considered in the analysis of the net oxygen production rates (Appendix-Fig.4). The average (±SD) of all α_P (rel. unit) extracted at decreasing irradiance (0.19 ± 0.12)

was 99% lower ($p<0.05$) than at increasing irradiance (20.04 ± 12.91). No diurnal trend for α_P was observed (data not shown). Production rates of *Microcystis* exposed to higher light intensities in the decreasing order were very scattered, often very low or negative and rarely saturated. Thus, extraction of P_{max} at decreasing light levels could have been made only for 9 cycles. Extraction of P_{max} at increasing light levels could have been made for the first 8 hours of incubation. Because of missing P_{max} data at decreasing irradiance levels, quantification of

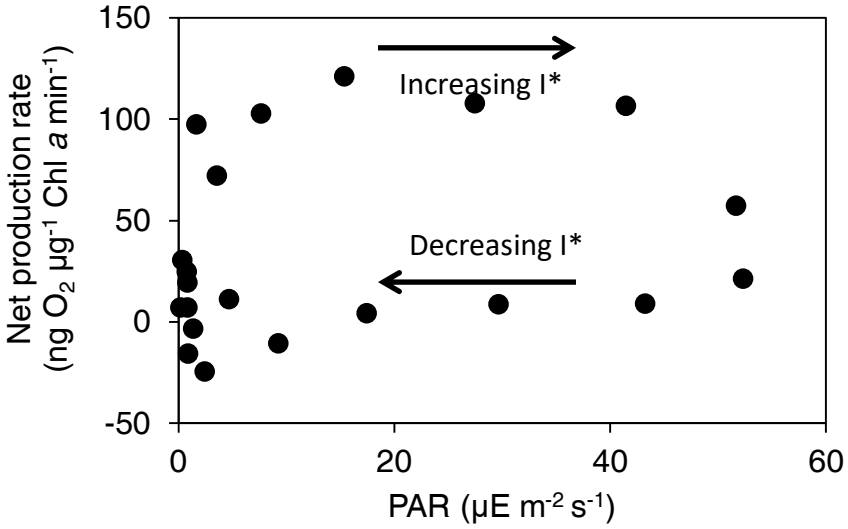


Figure 1. Example of duplicates averaged net oxygen production rates calculated every minute in a 20min light cycle (here between 20-40min incubation). Arrows represent the direction of the irradiance levels I*.

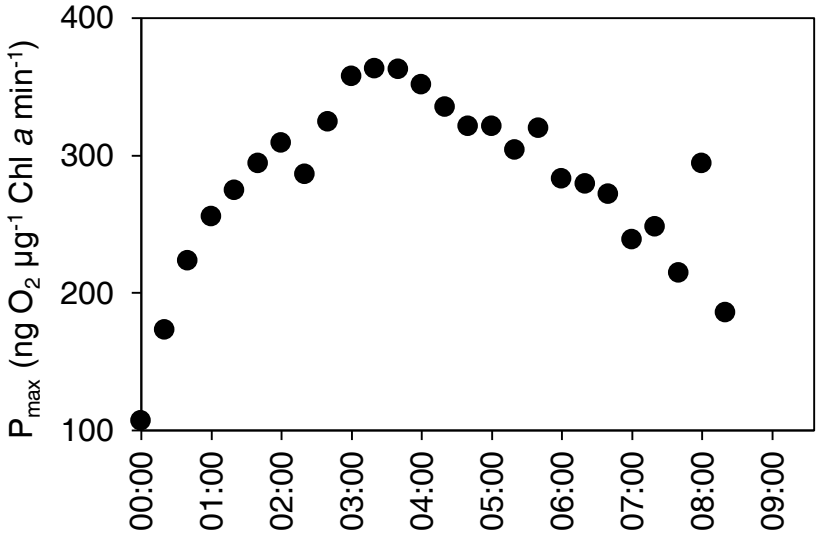


Figure 2. Diurnal evolution (12:12 photoperiod) of the maximal production rate P_{max} extracted at increasing irradiance levels under fluctuating light.

the short-term hysteresis by the P_{max} variation ($P_{max,increasing\ I^*}$ - $P_{max,\ decreasing\ I^*}$) could not be performed. Nonetheless, the average (±SD) of all P_{max} (ng 0_2 μg^{-1} Chl *a* min^{-1}) extracted at decreasing irradiance (67.8 ± 58.9) was 76% lower ($p<0.05$) than at increasing irradiance (283.4 ± 65.7). Maximal production rates P_{max} of *Microcystis* exposed to increasing light levels followed a diurnal trend, with a period of increase lasting for the first 4 hours of incubation to fluctuating light (Fig.2). Interestingly, decline in P_{max} between increasing and decreasing light levels occurred at short timescale within the 20min light cycles but also at the daily timescale (Fig. 3). Indeed, P_{max} at daily increasing irradiance levels (from t0 to t6) were higher than at the same mean light intensity (averaged PAR received during a 20min cycle) but in the decreasing order (from t6 to t12). The decline in P_{max} started after 4 hours incubation.

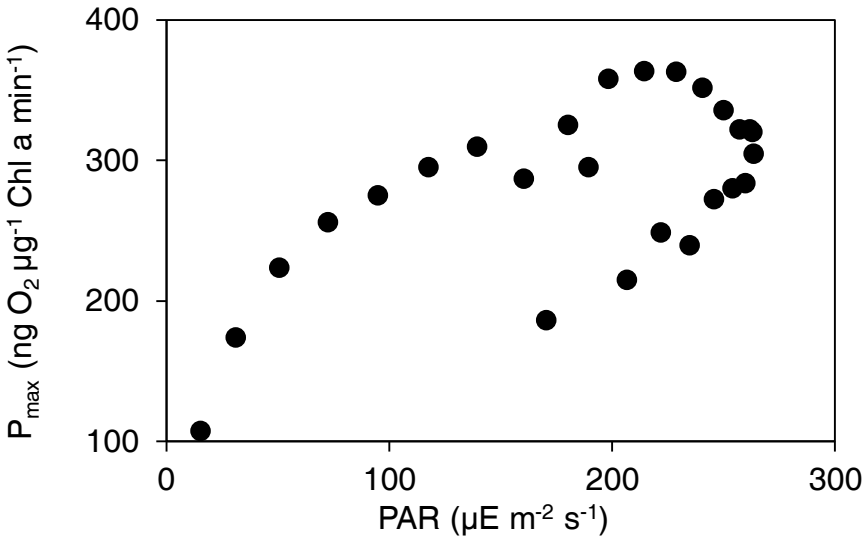


Figure 3. Light-dependency of the maximal production rates P_{max} extracted at increasing irradiance levels under fluctuating light.

The hysteresis in the net oxygen production rates occurring within the 20min light cycles also affected the compensation light intensity PAR_{compP}. The average (±SD) of all PAR_{compP} (µE m^{-2} s^{-1}) extracted at increasing irradiance (6.7 ± 4.8) was 98% lower ($p<0.05$) than at decreasing irradiance (320.5 ± 240.6). For the 8 first hours of incubation, compensation light intensity PAR_{compP} extracted at increasing and decreasing irradiance levels linearly increased with time (Fig.4). More interesting, P_{max} at increasing irradiance levels and PAR_{compP} at decreasing irradiance levels of the same 20min cycle were linearly related (Fig.5; P_{max} = 0.58*PAR_{compP} + 212.7, R^2=0.62, $p<0.001$). This relationship was only true for the first 4 hours of incubation,

during the diurnal increase of P_{max} (Fig.2). After 4 hours incubation, the relationship seemed to reverse but was not significant ($p>0.05$).

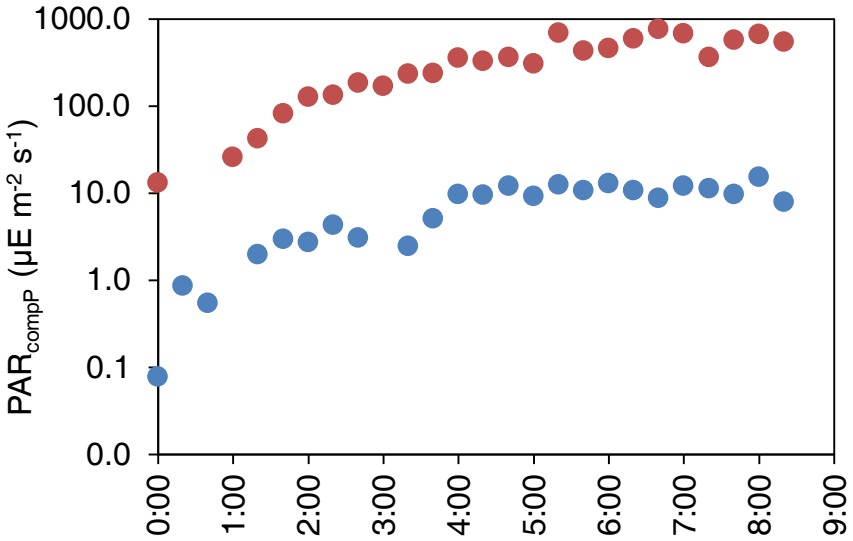


Figure 4. Diurnal evolution of the compensation light intensities PAR_{compP} (logarithmic scale) extracted at decreasing (red dots) and increasing (blue dots) irradiance levels under fluctuating light.

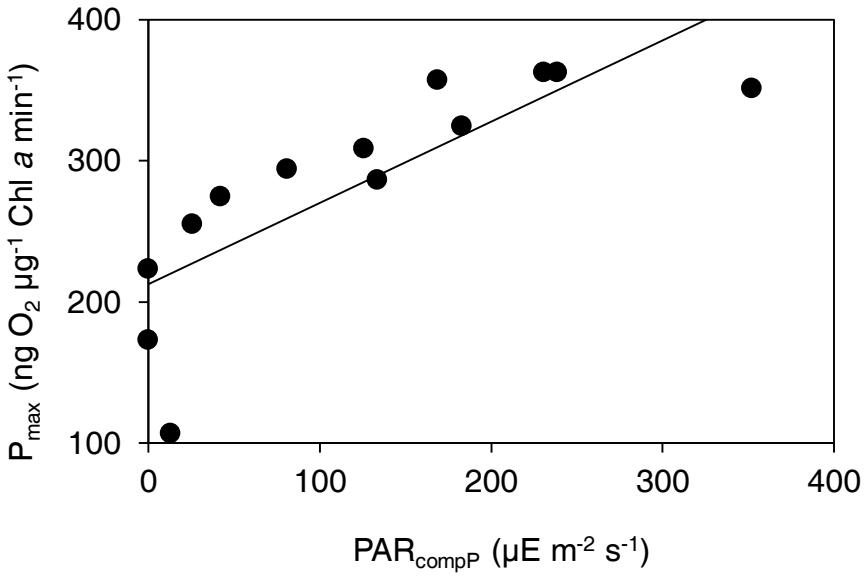


Figure 5. Relationship between P_{max} at increasing irradiance levels and PAR_{compP} at decreasing irradiance levels of the same 20min light cycle. Only data for the four first hours of incubation are presented.

Discussion

We observed higher ETR_{max} of *Microcystis* incubated under fluctuating light compared to those incubated under constant light supply. Moreover, diurnal increase of the ETR_{max} occurred only under fluctuating light. This enhancement of potential photosynthesis under fluctuating light supply has been already reported in laboratory conditions for cyanobacteria monocultures (Kana and Glibert, 1987; Shatwell et al., 2012) and under semi-natural conditions for lake communities (Köhler et al., 2018; Guislain and Köhler, unpubl.). Higher maximal photosynthetic rate of mixed algae allow them to better exploit intermittent exposure to surface high light intensities while limiting the risk of photo-inhibition (Cullen and MacIntyre, 1998). It is probably for this reason that we observed no photosynthesis enhancement at low light intensities under fluctuating light supply. Indeed, increase in α_{ETR} of algae incubated under fluctuating light may occur but remains much less reported (Nicklisch and Fietz, 2001; Shatwell et al., 2012).

Electron transport rate is one of the fastest dynamic responses of phytoplankton to changing light (Ferris and Christian, 1991a). For both light treatments, we observed no short-term variation in photosynthetic traits of *Microcystis* population between increasing and decreasing irradiance levels of the same intensity. Photosynthesis of algae, acclimated or not to fluctuating light exposure, was thus not affected by 20min cyclic light intensity variations. However, at the same timescale than of the ETR measurements (single minute), we observed an hysteresis in the net oxygen production rates of algae acclimated to fluctuating light conditions. This hysteresis was defined by a strong decrease in P_{max} and α_P when light was supplied in the decreasing order compared to in the increasing order. Diurnal hysteresis in oxygen production was also observed in our investigation and has been already documented (*e.g.* Harris and Lott 1973; Falkowski and Owens, 1978). But, to our knowledge, it is the first time that such hysteresis is observed at a single minute timescale for algae incubated under fluctuating light. Processes invoked in the diurnal hysteresis may thus act at much shorter timescale under fluctuating light supply. Under nutrient- and CO_2-replete conditions and without any concomitant changes in photosynthesis, this rapid hysteresis should be only due to light-dependent increase in oxygen consumption by dark respiration and photorespiration (Levy et al. 2004). In addition, this hysteresis was always coupled with an increase in compensation light intensity for net production at decreasing compared to increasing irradiance levels (compensation light intensity for ETR was always null). An increase of

compensation light intensity PAR_{compP} without changes in photosynthesis reflects higher respiratory maintenance costs of algae (Falkowski and Owens, 1978; Falkowski et al., 1994). This strengthens our argumentation in favour of a hysteresis driven by light-dependent respiration losses under fluctuating light. In fact, respiration may be enhanced in the light (Beardall et al., 1994; Hotchkiss and Hall, 2014), probably in part because of more substrate for respiration in the light (Weger et al., 1989). Under fluctuating light, algae accumulate carbohydrates during intermittent light peaks (at increasing irradiance) and may mobilize these fresh photosynthetic products by rapidly enhancing respiration for maintenance and biosynthesis at decreasing irradiance (Geider and Osborne, 1989).

Several studies found a constant ratio between linear electron transport generated by photosystem II (PSII), as we measured in the PAM, and gross oxygen production only at subsaturating light intensities (*e.g.*Flameling and Kromkamp, 1998; Masojidek et al., 2001; Toepel et al., 2004). At high light, gross oxygen production often declined whereas ETR still increased or remained constant. The decline in gross oxygen production was ascribed to an increase in respiration in the light (*e.g.* Beardall et al., 1994; Weger et al., 1989), cyclic electron transport around PSII (*e.g.* Prasil et al., 1996), Mehler reaction (*e.g.* (Kana, 1993; Wagner et al., 2006b), or photorespiration. The latter was probably less important given the low oxygen concentrations during our measurements. The cyclic electron transport has been shown to be low for algae incubated under fluctuating compared to incubation under constant light (Wagner et al., 2006b). We hence expect the observed within-cycle variations in net oxygen production to be due for a great share to respiration, but an exact quantification of respiration during the photoperiod is not permitted from our results.

Another interesting point is the positive relationship between P_{max} and PAR_{compP} (Fig.5). Within 20min light cycles (and only during the diurnal increase of P_{max}), higher P_{max} at increasing irradiance resulted in higher PAR_{compP} at decreasing irradiance. PAR_{compP}, the light intensity when net production is null, is driven by the balance between photosynthesis at limiting light and maintenance respiration. Since we observed no changes in electron transport rate within cycles (at t0, t6 and t12), variations in PAR_{compP} for production was in our investigation only due to maintenance respiratory costs and depended on P_{max}. A similar dependency between growth at saturating light and compensation light intensity for growth has been observed for a lake phytoplankton community incubated at vertical mixing (Guislain et al., 2019b). Guislain et al. expected this relation to be due the enhancement of photosynthesis at high irradiance for biosynthesis, increasing de facto

maintenance respiratory costs at limiting light. In this investigation, we showed the clear tradeoff between production at high and at low irradiance for *Microcystis* experiencing 20min light cycles – the link being respiration. Here, the photosynthetic products built at saturating light (P_{max}) may be directly used in effective darkness for maintenance (PAR_{compP}). To our knowledge, this is the very first time that this tradeoff is observed at a single minute time resolution under fluctuating light. By extension, and as assumed by Guislain and colleagues (2019),the light-dependent respiration should be a pillar of the trade-off between production/growth at saturating and limiting light under fluctuating light exposure.

Conclusion

In the present study, we observed for the first time a production hysteresis at a single minute timescale for algae incubated under fluctuating light. Without within-light cycle acclimation of photosynthesis, this hysteresis was due to light-dependent increase in oxygen consumption at decreasing light intensities, probably by increasing dark respiration in the light. This increase in respiration is expected to parallel the diurnal increase in maximal production rates (and maximal electron transport rate). Under fluctuating light exposure, *Microcystis* probably accumulated photosynthetic products at increasing irradiance and mobilized these fresh resources by rapidly enhancing respiration for maintenance and biosynthesis at decreasing irradiance. Further, our investigation shows that the light-dependent respiration should be a pillar of the trade-off between production and thus growth at saturating and limiting light under fluctuating light.

Acknowledgements - This manuscript presents few results of a much larger experiment. We acknowledge Paul Genz, Berta Ortiz-Crespo and Julia Kionka who worked as student assistants. We thank Barbara Stein for HPLC analyses.

Funding – This work is part of the Dino-German water supply project SIGN funded by the German Ministry of Education and Research (Grant number 02WCL1336D).

Author Contributions

AG lead writer, designed and performed the experiment, analysed and interpreted the results. JK designed the experiment and commented on the manuscript.

Chapter 4

Manuscript title:

Variation in species light acquisition traits under fluctuating light regimes: implications for non-equilibrium coexistence.

Alexis Guislain[1,2], Beatrix E. Beisner[3,4] and Jan Köhler[1]

[1]Department of Ecosystem Research, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany.

[2]Department of Ecology and Ecosystem Modelling, University of Potsdam, Potsdam, Germany

[3]Groupe de Recherche Interuniversitaire en Limnologie et en Environnement Aquatique (GRIL) and Department of Biological Sciences, University of Québec at Montréal, Montréal, Québec, Canada.

[4]Department of Experimental Limnology, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Stechlin, Germany.

Published as:

Guislain, A., Beisner, B. E., and Köhler, J. (2019). Variation in species light acquisition traits under fluctuating light regimes: implications for non-equilibrium coexistence. *Oikos* 128, 716–728. doi:10.1111/oik.05297.

Abstract

Resource distribution heterogeneity offers niche opportunities for species with different functional traits to develop and potentially coexist. Available light (photosynthetically active radiation or PAR) for suspended algae (phytoplankton) may fluctuate greatly over time and space. Species-specific light acquisition traits capture important aspects of the ecophysiology of phytoplankton and characterize species growth at either limiting or saturating daily PAR supply. Efforts have been made to explain phytoplankton coexistence using species-specific light acquisition traits under constant light conditions, but not under fluctuating light regimes that should facilitate non-equilibrium coexistence. In the well-mixed, hypertrophic Lake TaiHu (China), we incubated the phytoplankton community in bottles placed either at fixed depths or moved vertically through the water column to mimic vertical mixing. Incubations at constant depths received only the diurnal changes in light, while the moving bottles received rapidly fluctuating light. Species-specific light acquisition traits of dominant cyanobacteria (*Anabaena flos-aquae*, *Microcystis spp.*) and diatom (*Aulacoseira granulata, Cyclotella pseudostelligera*) species were characterized from their growth-light relationships that could explain relative biomasses along the daily PAR gradient under both constant and fluctuating light. Our study demonstrates the importance of interspecific differences in affinities to limiting and saturating light for the coexistence of phytoplankton species in spatially heterogeneous light conditions. Furthermore, we observed strong intraspecific differences in light acquisition traits between incubation under constant and fluctuating light – leading to the reversal of light utilization strategies of species. This increased the niche space for acclimated species, precluding competitive exclusion. These observations could enhance our understanding of the mechanisms behind the Paradox of the Plankton.

Keywords: Fluctuating light, Light acquisition traits, Phytoplankton photoacclimation, Niche partitioning, Non-equilibrium coexistence.

Introduction

It is well recognized that spatial and temporal heterogeneity offer niche opportunities for species with different ecological strategies to develop and potentially coexist (Chesson and Case 1986, Chesson 2000). Spatial heterogeneity reduces niche overlap, enabling coexistence by favouring different species in different local environments through environmental filtering. Temporal heterogeneity can also promote species coexistence through differential nonlinear species-specific responses to a fluctuating limiting factor; different species dominating at times when they are able to most actively use the resource (Chesson 2000, Adler et al. 2013). Thus, the impact of environmental variability on organisms may lead to different species performances and community composition than those measured under constant conditions (Koussoroplis et al. 2017). Empirical work on the effects of resource heterogeneity on species diversity maintenance and competition has been done on animals and terrestrial plants (reviewed by Amarasekare 2003, Silvertown 2004). In aquatic ecology, the coexistence of several phytoplankton species in a seemingly homogeneous environment was originally characterized as the 'Paradox of the Plankton' (Hutchinson 1961).

As the major primary producers on Earth, phytoplankton are responsible for about half of the global net production of photosynthetic organisms (Field et al. 1998). Their community composition may greatly affect food webs and biogeochemical cycles (Falkowski et al. 1998, Litchman et al. 2015). Consequently, it is important to understand how environmental variation affects phytoplankton biodiversity. Phytoplankton have very short generation times (≈ 1 day), are very easy to culture and have readily measurable functional traits affecting fitness in a given environment. Thus, they provide ideal models to test the effects of spatio–temporal environment variability on organisms. Studies involving phytoplankton exposed to varying resource levels have focused primarily on the effects of fluctuating nutrient supplies on species composition both in the laboratory (Sommer 1984, 1985) and in nature (Beisner 2001). Light is another essential resource for phytoplankton growth. Increasing efforts have been made to better understand the effects of fluctuating intensities on phytoplankton physiology under controlled (Nicklisch 1998, Havelková-Doušová et al. 2004, Shatwell et al. 2012) and semi-natural conditions (Marra 1978, Köhler et al. 2018). Nevertheless, very few studies have focused on the effects of fluctuating light levels on species competition and coexistence (Litchman 1998, Flöder et al. 2002), solely

investigating species diversity and/or species-specific growth rates at either low or high light levels.

In nature, light availability for phytoplankton fluctuates on timescales ranging from milliseconds to seasons (Falkowski 1984, Ferris and Christian 1991). Short-term light fluctuations affect several physiological processes such as photosynthesis (MacIntyre et al. 2000, Fietz and Nicklisch 2002), respiration (Avendaño-Coletta and Schubert 2005) and consequently, growth (Shatwell et al. 2012, Köhler et al. 2018). Phytoplankton growth is nonlinearly related to light availability, with a proportional increase in the limiting range of light intensities, constant growth at saturating light intensities, and a transition region around the onset of growth saturation. From such growth–light relationships, one may extract demographic traits of a population that can be seen to represent light acquisition traits as they provide reliable indicators of the ability of one species to grow at certain light intensities (Litchman et al. 2012). Traits include: the initial slope of the growth–light curve (α) which reflects the growth efficiency at limiting light; the maximum growth rate at saturating light (μ_{max}); and the light intensity at zero growth (PAR_{comp}), the so-called compensation light intensity (Fig. 1).

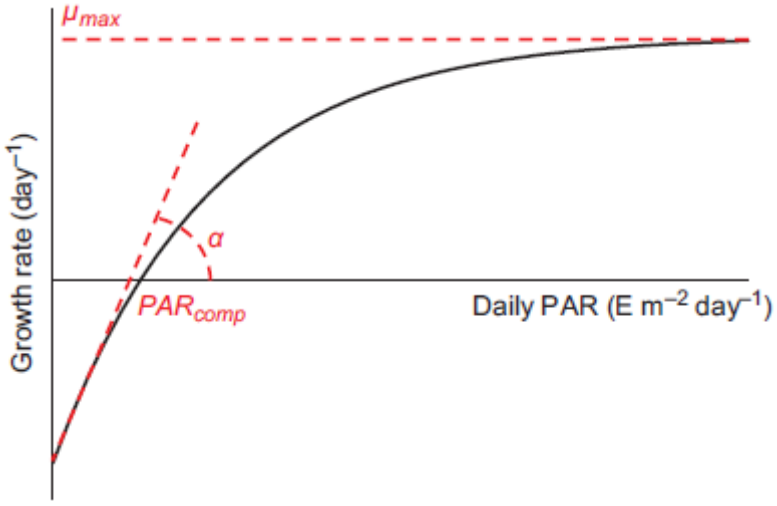


Figure 1. Graphical description of the light acquisition traits α, μ_{max} and PAR_{comp}.

These light acquisition traits calculated from traditional growth–constant light relationships measured in the laboratory have been used to explain phytoplankton distributions along environmental light gradients (Schwaderer et al. 2011). Assuming no co-limitation with other factors such as grazing or nutrient supply, a species with the higher α is expected to outcompete the others under limiting light levels. Conversely, a species with the

highest μ_{max} is expected to outcompete the others under saturating light levels. However, it has been shown that the light acquisition traits are plastic and may have different values between incubations under constant and fluctuating light (Shatwell et al. 2012, Köhler et al. 2018). This trait plasticity reflects the timescale-dependent ecophysiological acclimation processes of phytoplankton to changing light intensities (Falkowski 1984, Ferris and Christian 1991). The acclimation mechanisms are species-dependent (potentially even clonal-dependent, Kardinaal et al. 2007) and should thus alter interspecific competition, promote coexistence or exclude inefficient species in diverse phytoplankton communities (Litchman 1998, Flöder et al. 2002). For instance, a species that is the best competitor at a certain constant light supply could coexist or even be displaced by a species with higher performance under fluctuating light of the same mean intensity.

In general, it is still unknown how species light acquisition trait variation under fluctuating light may alter niche partitioning and thus species coexistence in bulk phytoplankton communities. We made the first attempt to fill this gap by investigating the effects of fluctuating light on light acquisition traits and relative biomass of dominant phytoplankton species from a diverse community under semi-natural conditions. We deliberately measured the light acquisition traits in a community context, and not for species cultured separately, because species generally diverge more in resource use to reduce niche overlap in a multispecies context (Lawrence et al. 2012). We mimicked vertical mixing and induced fluctuating light regimes by computer-controlled motion of subsamples from a lake phytoplankton community in the frequently mixed, turbid, hypertrophic Lake TaiHu (China). The investigated community was adapted to Lake TaiHu's temperature and frequent mixing. It was incubated under nutrient-replete conditions and drastically reduced grazing pressure. Thus phytoplankton dynamics were expected to be mostly driven by rapid acclimation to light climate treatments within a couple of days. We evaluated variation in light acquisition traits of phytoplankton between stratified and mixed conditions and used these to describe realized light niches, thereby improving understanding of non-equilibrium species coexistence under semi-natural conditions. We hypothesized that in this natural phytoplankton community: 1) fluctuating light would modify species-specific growth–light relationships and, as a consequence, light acquisition traits (α, μ_{max} and PAR_{comp}). A set of species light acquisition traits was considered here as a light utilization strategy. Following support of this first hypothesis, we then expected 2) a relative change in species biomass over the light gradient (limiting versus saturating light) and between subsamples incubated under

fluctuating light conditions relative to those experiencing constant light. We further hypothesized that 3) niche partitioning of the dominant species was possible over gradients of light and mixing depth in the water column.

Material and methods

Study site and experimental setup

Lake TaiHu (China, 31°14'N, 120°8'E) is a very large (2340 km^2), shallow (1.9 m mean depth), hypertrophic, turbid and wind-exposed lake. Due to the intensification of human activities in the catchment area, total nitrogen and phosphorus concentrations in the lake have been increasing since the 1980s and resulted in intensified blooms occurring more frequently (Duan et al. 2009, Qin et al. 2010). Cyanobacteria blooms can reach 1000 km^2 and may occur from May to November (Duan et al. 2009). The field experiment was conducted from 7 to 16 September 2016, during the development of the cyanobacteria bloom (mostly *Microcystis* spp.). Our experimental site was situated in the Meiliang Bay of Lake TaiHu (northern part of the lake) on top of the Nanjing Institute of Geography and Limnology (NIGLAS) landing, about 200 m offshore.

Prior to sampling, we removed any surface scums containing dying cyanobacteria cells and sampled lake water at 30 cm depth to recover the natural phytoplankton community. We filtered the water through a 100 µm sized mesh to remove large zooplankton, and then gently bubbled it with N_2 for five hours to kill any small remaining zooplankton by anoxia. We added 12× concentrated MIII-KS fresh culture medium, to obtain 1× final concentration (Nicklisch et al. 2008 for detailed composition). After re-aeration we distributed the lake water into 500 ml transparent incubation bottles (Teflon Fluorinated Ethylene Propylene, Nalgene). These bottles provide the best tradeoff between robustness for incubation in the lake and photosynthetically active radiation (PAR (400–700 nm)) and ultra-violet (UV-A (320–400 nm)) transmittance (mean transmittance to PAR = 72 ± 6.6%; to UV-A = 51.4 ± 3.5%).

We performed two identical experiments with regard to their design and methods, starting at sunrise each time and lasting either five days (7–11 September) and four days (13–16 September). No difference in species composition was noted between inocula at the two experimental periods. The species composition of the inocula was very diverse (n = 57 species) (Supplementary material Appendix 1 Table A1).

Bottles were installed in triplicate in transparent holders placed at fixed depths and vertically moved by a computer-controlled lift in the lake (method described in Köhler et al. 2018). Phytoplankton incubated at constant depth received only the natural sinusoidal diurnal course of sunlight, a treatment that we will refer to as constant light. In contrast, communities incubated in bottles moved vertically through the water column received fluctuating light, by superimposing the vertical light gradient on the natural sinusoidal diurnal sunlight. The lifts simulated a circular movement with 20 min per revolution, replicating to some extent the full overturn of typical Langmuir cells (Denman and Gargett 1983, Schubert and Forster 1997, Thorpe 2004). We fixed incubation bottles in triplicates at 0, 0.2, 0.4 and 0.8 m depth (constant light treatment). The moving bottles rotated between the water surface (0 m) and 0.5, 1.0 and 1.8 m depth (fluctuating light treatment). The daily PAR values received in both treatments are given in the Supplementary material Appendix 1 Table A2, Fig. A1.

Fully dark-adapted subsamples were taken each morning before sunrise. Sample volumes ranged from 80 to 100 ml to ensure similar total biomass between the different incubation bottles. To avoid nutrient limitation, we refilled the bottles with a mix of filtered lake water (Whatman GF/F glass microfiber) and 12× concentrated MIII-KS fresh culture medium, to obtain 1× final concentration. The bottles were re-incubated in the lake within 20 min.

Abiotic conditions

Global radiation data were measured using a 2π light sensor type and were obtained from the NIGLAS monitoring station (TaiHu Laboratory for Lake Ecosystem Research TLLER) located near the experimental site. To obtain daily PAR intensities, we first corrected the global radiation for light attenuation in the lake following the Lambert–Beer's law:

$$I_z = I_0 * e^{-kz}$$

where *Iz* is the light intensity at depth *z* (m), *Io* is PAR at the water surface and *k* the light attenuation coefficient (m^{-1}). The latter was calculated from daily light measurements at 0.5 m intervals from the surface to 1.5 m depth with a spherical spectroradiometer. Then, we corrected the light data for shade produced by the pier (when applicable), for wavelength-

specific transmittance of the incubation bottles and the actual vertical position of the moved phytoplankton.

Vertical profiles of temperature were measured every 5 min using temperature loggers sealed to the bottles holders. The lake was very well mixed with temperatures between the lake surface and the bottom differing by less than 0.36°C on average during the experimental period.

Cell counts

Species composition was monitored at the beginning, after two days and at the end of each of the two experiments. Subsamples were fixed in Lugol's solution (Throndsen 1978). Subsamples taken from each replicate were mixed together to reduce the number of samples to count. Cell abundances of the dominant phytoplankton species were obtained after counting at least 400 algal objects (cell, filament or colony) (Lund et al. 1958) per sample by inverted microscopy following the Utermöhl method (Utermöhl 1958). Cell volumes were measured from at least 20 individuals of each species from any sample under the same microscope using ImageJ software. Biovolumes (proxy for phytoplankton biomass) were calculated by multiplying averaged cell volumes by cell abundances. We measured biovolumes for 15 different species: seven cyanobacteria, five diatoms and three chlorophyceae.

Data analysis

The biovolume of a species i relative to the biovolume of the group it belongs to was calculated after two days and at the end of both experiments as:

$$\text{Relative biovolume}_{\text{species i}} = \frac{\text{biovolume}_{\text{species i}}}{\text{biovolume}_{\text{group}}}$$

Daily species-specific growth rates μ_i (day^{-1}) were calculated as follows, accounting for daily dilution:

$$\mu_i = \frac{\ln\left[\frac{\text{biovolume}_{t1} * \text{vol}}{\text{biovolume}_{t0} * (\text{vol} - \text{vol}_{\text{dilution}})}\right]}{t}$$

with $biovolume_{t0}$ and $biovolume_{t1}$ being the biovolumes of species i at times t0 and t1. *Vol* is the total volume of the incubation bottle and $vol_{dilution}$ is the volume sampled for analysis and replaced with fresh culture medium.

To build the growth–light relationships, we fit nonlinear mixed effects models to the observed growth rates using the model of Webb et al. (1974):

$$\mu = \mu_{\max}\left[1 - \exp\left(\frac{-\alpha\left(\mathrm{PAR} - \mathrm{PAR}_{\mathrm{comp}}\right)}{\mu_{\max}}\right)\right]$$

where μ_{max} is the growth rate at saturating light (day^{-1}), α is the growth efficiency at limiting light ($m^2\ E^{-1}$), PAR_{comp} is the compensation light intensity ($E\ m^{-2}\ day^{-1}$) and *PAR* is the daily PAR exposure ($E\ m^{-2}\ day^{-1}$).

The daily PAR exposure was averaged over (day 0–1) when plotting growth rates measured at day two and averaged over (day two–end experiment) when plotting growth rates measured at the end of the experiment. We obtained the estimates of the light acquisition traits μ_{max}, α and PAR_{comp} (± SE) for the best fitting model.

To obtain reliable trends along the light gradients and improve parameter estimations of the effects of light fluctuations on non-equilibrium species coexistence and phytoplankton physiology, we opted for counting more samples along the daily PAR gradient over more replicates at fewer light intensities. This strategy is in line with the recent call for 'regression-based experimental designs' expressing the need to increase the number of predictor levels while decreasing the number of replicates (Cottingham et al. 2005, Beier et al. 2012, de Boeck et al. 2015, Schweiger et al. 2016). Schweiger et al. (2016) recently provided methodological recommendations for such a protocol, arguing that where greater systematic error is likely, such as in field studies, continuous sampling without replication is preferable to sampling fewer but replicated predictor levels along the same gradient.

Realized species niches to daily PAR and mixing depth gradients

In addition to estimating the relative biovolumes of dominant species of cyanobacteria and diatoms over a gradient of daily constant and fluctuating PAR, we also wanted to describe the effects of the magnitude of light fluctuations on phytoplankton composition. To this end, we examined species dominance or coexistence regions of diatoms and cyanobacteria over gradients of mixing depths and daily PAR exposure. Traditionally, one would examine the

equilibrium phytoplankton growth. But stable growth over time is usually only achievable in laboratory experiments. Given that our study monitored a whole community under natural conditions with diurnal light variation, we cannot expect phytoplankton species to be adapted to a given daily PAR. Thus, we defined regions of 'major contribution relative to other species'. One species was declared the 'winner' over a second species if the difference between their relative biovolumes was >10% (an arbitrary but useful threshold). Species 'coexisted' when the variation around their relative biovolumes was ≤10%. This approach does not describe steady-state species composition but instead describes the short-term niche partitioning over the daily light supply and mixing depth gradient.

We investigated how species within each group (diatoms or cyanobacteria) could coexist in situ through their response to light conditions, because it is in these groups that species are likely to compete more severely for light. Prokaryotes (cyanobacteria) and eukaryotes (diatoms) differ in many aspects of their cellular components, physiology, evolutionary history and acclimatization potential (Glover et al. 1987, Gregory 2001, Yoon et al. 2004, Schwaderer et al. 2011) that should promote greater differences in light use between than within groups (Schwaderer et al. 2011).

Statistical analyses

Nonlinear mixed effects models were implemented with the nlme R package (Pinheiro et al. 2018 – library nlme R package ver. 3.1-137) with maximum log likelihood and setting 'incubation bottle' as random factor to account for temporal autocorrelation of growth measurements and ensure independence of errors.

Differences in the light acquisition traits (μ_{max}, α and PAR_{comp}) between constant and fluctuating light were assessed using the nonlinear Webb model (Webb et al. 1974) with 'incubation bottle' as random factor. We tested the null hypothesis that the light acquisition traits did not vary between constant and fluctuating light, against the alternative hypothesis that one or more traits did vary between treatments. Conclusions on treatment effects were based on model comparisons with F-tests following Bates and Watts (1988, p. 105ff) and providing p-values. The models selected were also supported by the lowest Akaike information criterion (AIC) (Akaike 1974; results not shown). We used the same analytical approach to assess the interspecific differences in the light acquisition traits (μ_{max}, α and PAR_{comp}) under constant and fluctuating light. Relative species biovolumes along the daily PAR gradient were fit by a logarithmic function (coefficient × PAR + intercept) using the nls()

command. Interspecific differences after two days of experiment were assessed by the same method.

All analyses were performed with R ver. 3.3.2 (<www.r.project.org>).

Data deposition

Data available from the Dryad Digital Repository: < http:// dx.doi.org/10.5061/dryad.2rh61qk > (Guislain et al. 2018).

Results

Light affinities of dominant species

At all times and in all treatments four taxa, the cyanobacteria *Anabaena flos-aquae* and *Microcystis* spp. and the diatoms *Aulacoseira granulata* and *Cyclotella pseudostelligera*, dominated the assemblages (85.3 ± 9.2% and 84.3 ± 4.3% of the total biovolume under constant and fluctuating light respectively). For convenience we will refer to these phytoplankton taxa by their genus names. *Anabaena* and *Microcystis* combined accounted for 25.5 ± 10.8% and 24.4 ± 9.3% of the total biovolume during the entire experimental period under constant and fluctuating light respectively. *Aulacoseira* and *Cyclotella* combined accounted for 59.8 ± 16.5% and 59.9 ± 9.6% respectively under constant and fluctuating light. The contributions of the main phytoplankton groups to the total biovolume are given in the Supplementary material Appendix 1 Table A3.

The contributions of diatoms to the total biovolume tended to slightly decrease with increasing daily PAR supply for the benefit of cyanobacteria (PAR effect not significant; $p > 0.05$) (Supplementary material Appendix 1 Fig. A2). Chlorophyceae were always very sparse. We noted no differences in the contribution of the main phytoplankton groups (diatoms, cyanobacteria and chlorophyceae) between constant and fluctuating light exposure (all p-values >0.05). Nevertheless, we observed a strong light dependency of the relative contributions of species within diatoms and cyanobacteria.

Figure 2 depicts species-specific growth–light relationships of the two dominant cyanobacteria (*Anabaena*, *Microcystis*) and the two diatoms (*Aulacoseira*, *Cyclotella*) under constant and fluctuating light (Supplementary material Appendix 1 Fig. A3 for intraspecific variation). The growth–light relationships of *Anabaena* and *Microcystis* intersected under both constant and fluctuating light because of different light affinities of each species to

limiting and saturating light. Under constant light (Fig. 2A), *Anabaena* had slightly higher growth rates at saturating light than did *Microcystis*, but lower growth rates at limiting light. Under fluctuating light (Fig. 2B) the strategies of both species were reversed with *Microcystis* having higher growth rates at saturating light than *Anabaena*, but lower growth rates at limiting light. Amongst the diatoms, *Cyclotella* always grew far better than *Aulacoseira* at saturating light (Fig. 2C, D). At limiting light, drastic differences in growth rates between species occurred only under mixed conditions, as *Aulacoseira* grew better than *Cyclotella*.

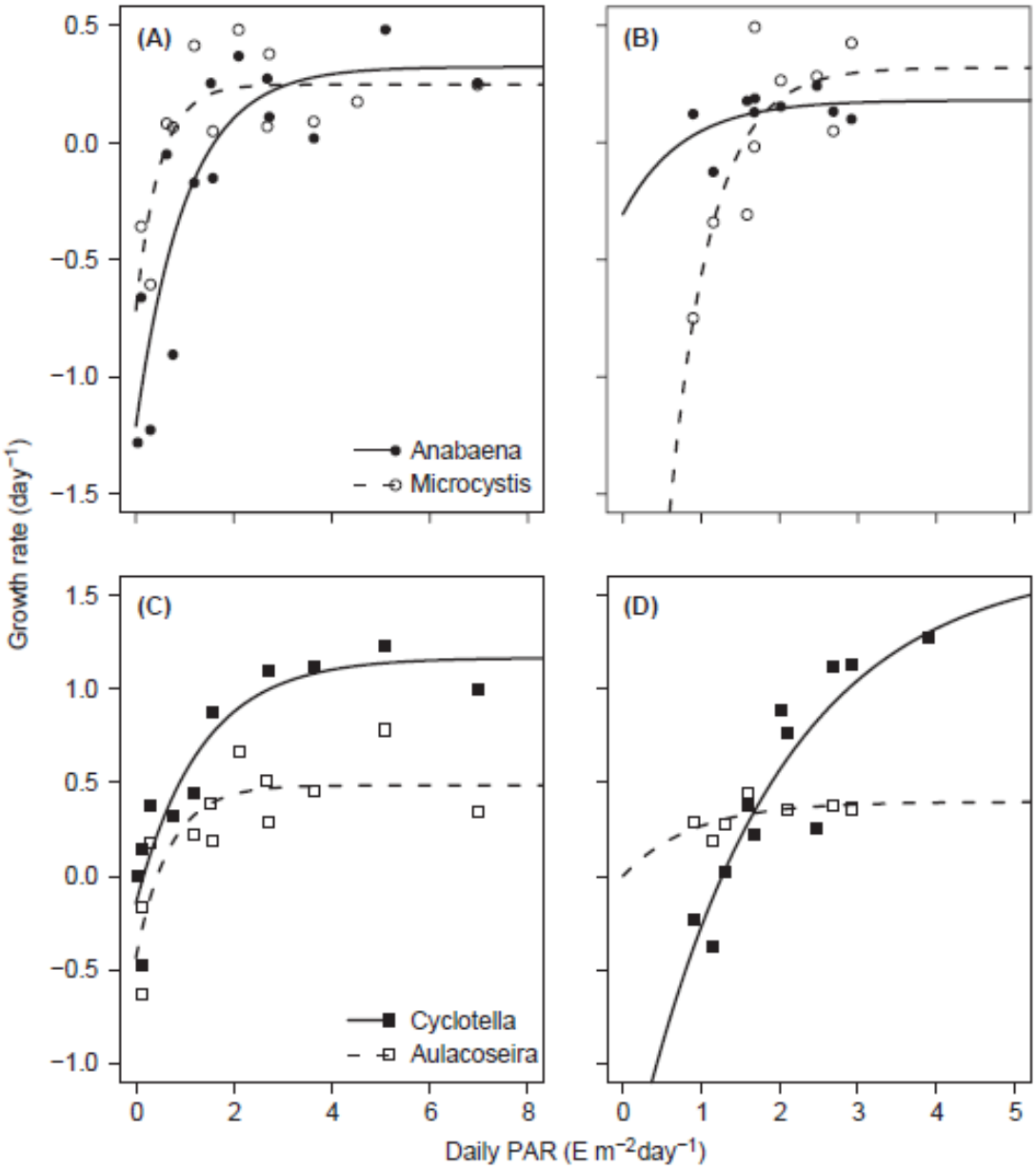


Figure 2. Species-specific growth–light relationships of the two dominant cyanobacteria (*Anabaena*, *Microcystis*) under (A) constant and (B) fluctuating light; and the two dominant diatoms (*Cyclotella*, *Aulacoseira*) under (C) constant and (D) fluctuating light.

Estimated values (± SE) of α, μ_{max} and PAR_{comp} of species dominating the phytoplankton community are presented in Table 1.

For cyanobacteria, lower growth rates at limiting light were linked to higher values of PAR_{comp}. *Anabaena* had significantly higher PAR_{comp} than *Microcystis* under constant light ($p < 0.01$). The opposite was true under fluctuating light ($p < 0.01$). Under constant light, *Anabaena* attained slightly higher μ_{max} than *Microcystis*, but needed a higher PAR_{comp} than under fluctuating light. Conversely, under fluctuating light, *Microcystis* had higher μ_{max} than *Anabaena* but needed a significantly higher PAR_{comp} than under constant light ($p < 0.001$). Growth efficiencies (α) did not drive the differences in growth rates between species as *Microcystis* always had higher α than *Anabaena* under both light exposures. Note that this trait increased slightly with positive intraspecific variation in μ_{max} and PAR_{comp}.

Table 1. Calculated light acquisition traits α, μ_{max} and PAR_{comp} (estimate ± SE) of the four dominant species under (A) constant and (B) fluctuating light. The goodness of fit is also presented for each trait in brackets. Units: μ_{max} in day^{-1}, α in $m^2\ E^{-1}$ and PAR_{comp} in $E\ m^{-2}\ day^{-1}$.

	Anabaena	*Microcystis*	*Aulacoseira*	*Cyclotella*
A				
μ_{max}	0.32 ± 0.18 (0.11)	0.25 ± 0.08 <0.05	0.48 ± 0.11 <0.01	1.16 ± 0.16 <0.001
α	0.31 ± 0.12 <0.05	0.52 ± 0.25 (0.08)	0.71 ± 0.43 (0.15)	0.88 ± 0.30 <0.05
PAR_{comp}	1.60 ± 0.39 <0.01	0.65 ± 0.23 <0.05	0.44 ± 0.20 (0.08)	0.16 ± 0.12 (0.25)
B				
μ_{max}	0.18 ± 0.08 (0.08)	0.32 ± 0.20 (0.19)	0.40 ± 0.07 <0.05	1.69 ± 0.84 (0.09)
α	0.24 ± 0.27 (0.43)	0.60 ± 0.23 (0.06)	0.44 ± 0.17 (0.08)	0.94 ± 0.29 <0.05
PAR_{comp}	0.76 ± 0.87 (0.43)	1.54 ± 0.22 <0.01	Set to 0	1.27 ± 0.13 <0.001

Amongst the diatoms, *Cyclotella* grew significantly faster at saturating light than *Aulacoseira* under both constant ($p < 0.001$) and fluctuating ($p < 0.001$) light (Table 1). In contrast to the cyanobacteria, higher μ_{max} of *Cyclotella* than of *Aulacoseira* was linked to higher PAR_{comp} under fluctuating light ($p < 0.001$) but not under constant light ($p > 0.05$).

To support the increase of its μ_{max} under fluctuating light, *Cyclotella* needed a significantly higher PAR_{comp} ($p < 0.001$) than under constant light. The three light acquisition traits of *Aulacoseira* slightly increased under constant light ($p > 0.05$).

As for the cyanobacteria, growth efficiencies (α) did not drive the differences in growth rates between species, as *Cyclotella* always had higher α than *Aulacoseira* under both light exposures. Note that this trait also increased with positive intraspecific variations of μ_{max}

and PAR_{comp}. In addition, compensation light intensities of both diatoms were almost always lower and α and μ_{max} almost always higher than for the cyanobacteria species.

Relative biovolumes of dominant species over the daily PAR gradient

The relative biovolumes of the two dominant cyanobacteria depended greatly on the daily PAR (Fig. 3A, B) and were significantly different between species (all p-values <0.05). Similar to the growth–light relationships that were measured in the same species community context, the fits of relative biovolumes intersected (Fig. 3A). *Anabaena* contributed more at constant saturating light, following its higher μ_{max} under such conditions. On the other hand, a lower PAR_{comp} and higher α enabled *Microcystis* to dominate at constant limiting light.

The incubation of the same initial community under fluctuating light reversed, after two days only, the relative biovolumes observed under constant light, reflecting the changes in light acquisition traits of both species between the two light exposures (Fig. 3B). *Microcystis* was the saturating light specialist under fluctuating light, increasing its contribution to the assemblage with fluctuating light intensities. *Anabaena* clearly dominated at fluctuating limiting light following its lower PAR_{comp} under such conditions.

PAR_{comp} values of the dominant cyanobacteria species clearly determined their relative contributions to the assemblage at limiting light. *Microcystis* always grew more efficiently (higher α) than did *Anabaena* under constant or fluctuating limiting light (Table 1). Yet, *Microcystis* dominated the assemblage only at constant limiting light (Fig. 3A). Nevertheless, at saturating light under both light treatments, the differences in relative biovolumes of the cyanobacteria were less pronounced (Fig. 3A, B). Note that the differences in light-dependent relative biovolumes were larger after five days (not shown because of the time dependence of biovolumes measured after two days and at the end of the experiments).

Unlike the cyanobacteria, the relative biovolumes of the diatoms along the gradient of daily PAR followed a similar pattern under both constant and fluctuating light (Fig. 3C, D) and were significantly different between species (all p-values <0.05). This result reflected the consistency of light affinities between constant and fluctuating light: *Cyclotella* always had higher μ_{max} than *Aulacoseira* under both constant and fluctuating light (Table 1). Therefore, the contribution of *Cyclotella* increased with increasing daily PAR supply. Differences in relative biovolumes of diatoms were more pronounced under fluctuating light and were described by higher μ_{max} and PAR_{comp} of *Cyclotella* under fluctuating light than under

constant light. As for the cyanobacteria, differences in light-dependent relative biovolumes were more pronounced after five days (data not shown).

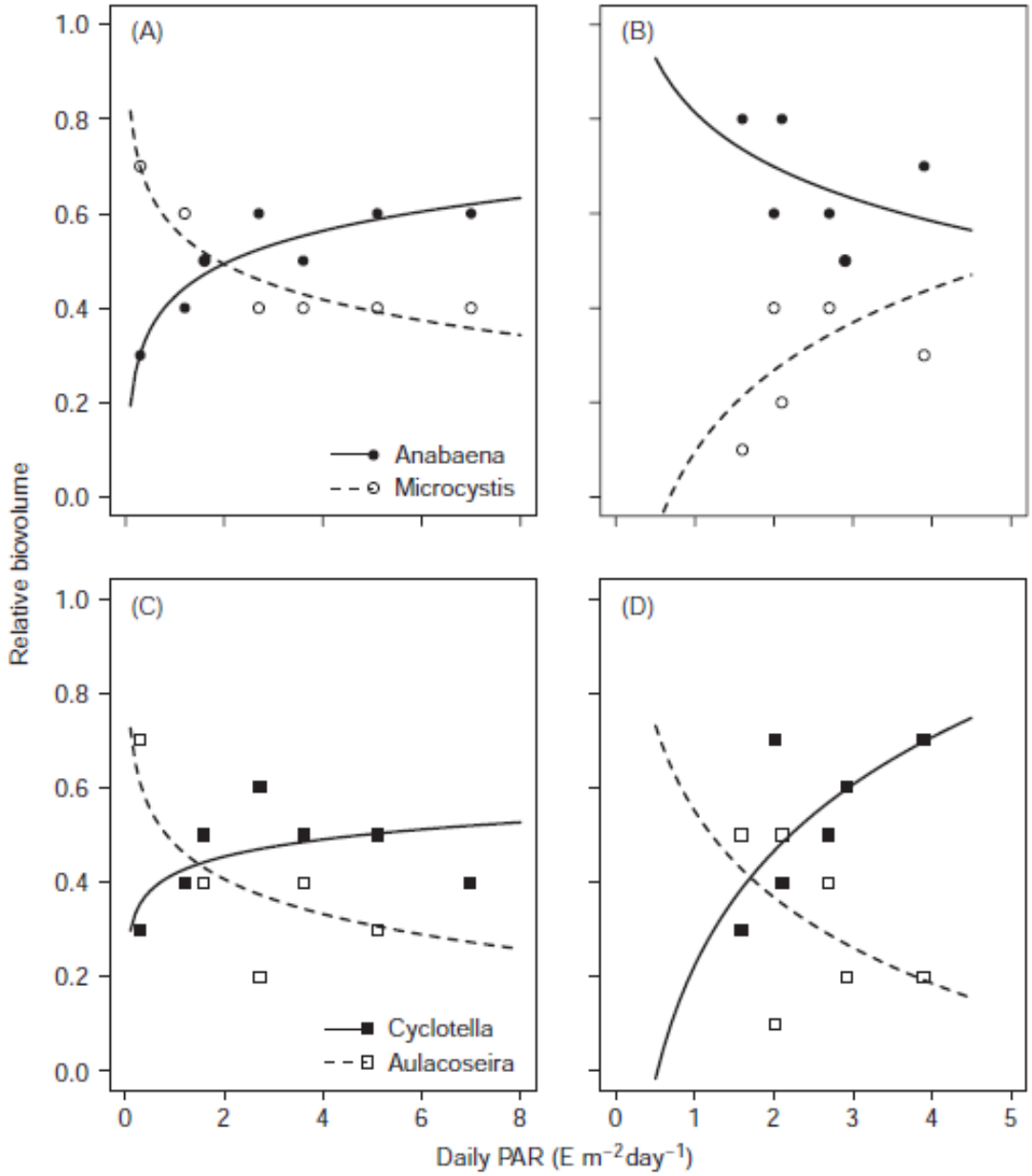


Figure 3. Light-dependency of the relative biovolumes of *Anabaena* and *Microcystis* to the cyanobacteria biovolume (A, B) and of *Cyclotella* and *Aulacoseira* to the biovolume of diatoms (C, D) under constant (A, C) and fluctuating light (B, D). Only relative biovolumes after two days of experiment are depicted.

Realized light niches over the daily PAR and mixing depth gradients

Realized light niches of cyanobacteria species were partitioned on both the daily PAR and mixing depth gradients (Fig. 4A). Under stagnant conditions, *Microcystis* dominated the

cyanobacteria biovolume at limiting light whereas *Anabaena* dominated at saturating light levels above 5 E m^{-2} day^{-1}. Under mixing conditions, *Anabaena* dominated the cyanobacteria assemblage at all investigated daily light intensities when the mixing depth was higher than 0.5 m. Finally, *Anabaena* and *Microcystis* equally contributed to the cyanobacteria community roughly at a daily light supply ranging from 2 to 5 E m^{-2} day^{-1} under stagnant conditions. Under mixing conditions, both species contributed equally at shallow mixing (0.5 m mixing depth).

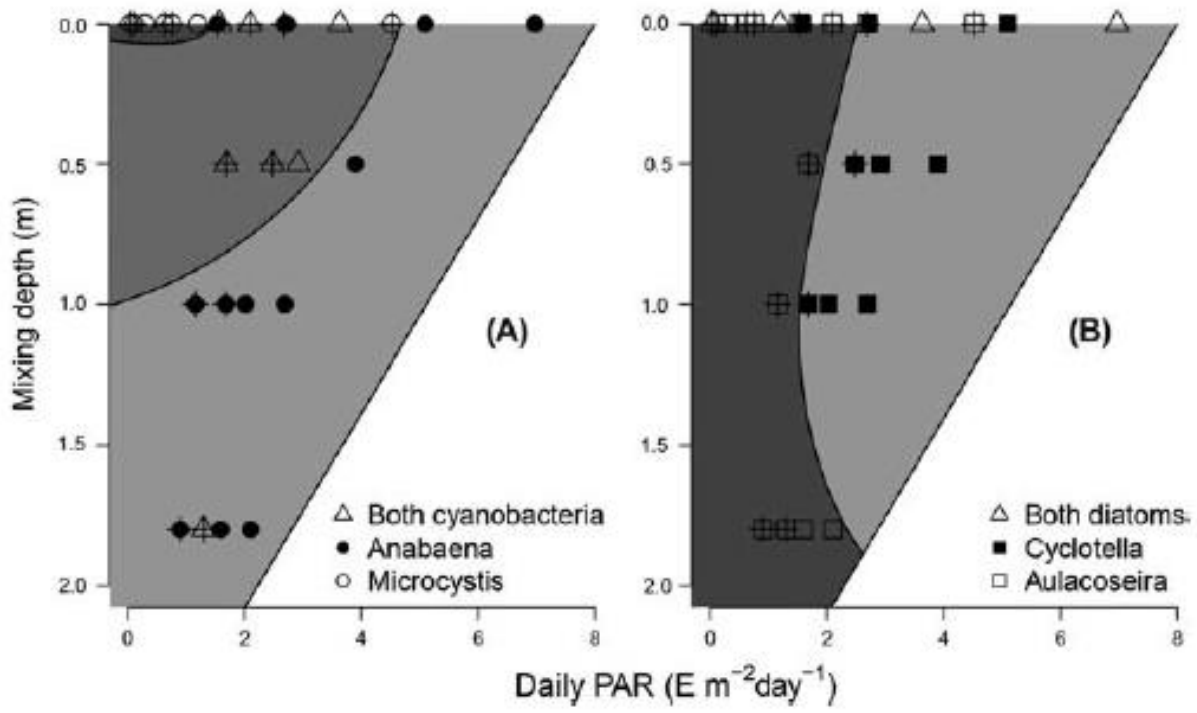


Figure 4. Realized niches of the (A) cyanobacteria and (B) diatoms after two days and at the end of the experiments (crossed symbols) over gradients of daily PAR exposure (E m^{-2} day^{-1}) and mixing depth (m).

Unlike the cyanobacteria species, the diatoms maintained consistent light utilization strategies under constant and fluctuating light (Fig. 2C, D, Table 1). Realized niches were thus determined only by the daily PAR gradient (Fig. 4B). *Aulacoseira* dominated over *Cyclotella* under stagnant and mixed conditions at low daily PAR. In contrast, when the daily PAR supply was greater than roughly 2 E m^{-2} day^{-1}, *Cyclotella* dominated over *Aulacoseira* regardless of mixing conditions.

Discussion

Mechanistic linkage between physiological processes and community dynamics

Light acquisition traits capture important aspects of the ecophysiology of phytoplankton (Litchman 2007), offering a promising mechanistic link between the environment and community dynamics in both marine (Edwards et al. 2013a) and freshwater (Edwards et al. 2013b) ecosystems. However, most studies to date used data obtained from traditional growth–light experiments performed in the laboratory and under constant light exposure, de facto underestimating the importance of light acquisition traits variation towards fluctuating light in nature (Nicklisch 1998, Shatwell et al. 2012).

The light acquisition traits we focused on (light–saturated growth μ_{max}, growth efficiency at limiting light α and compensation light intensity PAR_{comp}) integrate many underlying physiological processes that are sensitive to light levels. μ_{max} and α are mainly driven by the energy allocated to growth (e.g. ribosomes) and light-harvesting machinery (*e.g.* chlorophyll complexes (Chla:C ratio) and accessory pigments) respectively (Langdon 1988, Klausmeier et al. 2004, Litchman 2007, Talmy et al. 2013). PAR_{comp}, the light intensity when $\mu = 0$, is driven by the balance between photosynthesis (and thus, light-harvesting machinery) at limiting light and maintenance respiration (Langdon 1988). PAR_{comp} is primarily affected by maintenance respiratory costs (Langdon 1988). Respiration consumes oxygen in the production of ATP and NADPH to support biosynthesis and cell growth (reviewed by Ferris and Christian 1991). As a consequence, the respiration maintenance to growth ratio is higher for high-light acclimated, fast-growing species (high μ_{max}) than for low-light acclimated species. Fast-growing species achieve compensation levels at higher light intensities and are thus less competitive at limiting light (Geider and Osborne 1989, Geider et al. 1996, Dubinsky and Stambler 2009). Also, excessive photosynthetic excitation may damage the photosystems that could result in additional respiratory costs (Richardson et al. 1983).

These light acquisition traits are inherently plastic and their values define the potential of species to grow at certain light supply. The light-saturated growth μ_{max} reflects the affinity for saturating light and a species with high μ_{max} is considered to be an opportunist, growing faster when light levels increase. On the other hand, a species with high growth efficiency at limiting light (α) and low compensation light intensity (PAR_{comp}) has low

light requirements and is considered as gleaner (Grover 1990, Litchman and Klausmeier 2008).

Because of the limited energy that can be devoted to the acquisition of a particular resource, physiological tradeoffs are expected between the light acquisition traits, such as between maximum growth rate (at saturating light) and growth efficiency (at limiting light) (Litchman and Klausmeier 2008). Therefore, one species may outcompete another at saturating or limiting light if its trait value offers a better overall performance. In our study, high μ_{max} always (under both constant and fluctuating light) described competitive dominance at saturating light levels. In contrast, species with low PAR_{comp} were more efficient at limiting light and almost always dominated their group biovolume under such conditions. The growth efficiency (α) has been used to characterize the affinity of a species when light is limiting (Schwaderer et al. 2011, Edwards et al. 2013a, b, 2015). Our study demonstrates that PAR_{comp} was the most relevant trait related to the ability of a species to outcompete others under constant and fluctuating limiting light supply. According to our results, the dominant species at limiting light was almost always the one with the lowest PAR_{comp} value, regardless of α. We expect that this may result from the short duration of our experiment as maintenance costs, such as photoprotection mechanisms (influencing PAR_{comp}) could act at shorter timescales than growth (determined by α at limiting light) (Falkowski 1984, Ferris and Christian 1991, MacIntyre et al. 2000). By measuring the species dominance patterns after only couple of days, we increased the relative importance of short-term mechanisms and likely favoured species with low PAR_{comp} rather than high α under limiting light. It is likely that α values could have had greater impact on competitive outcomes at limiting light on longer timescales. However, longer periods of constant conditions rarely occur in dynamic systems.

Overall, the short-term gleaner–opportunist tradeoff exhibited by species in our study seemed to be driven by the enhancement of photosynthesis that increases slightly α, and to a much larger extent μ_{max} – increasing de facto the maintenance respiratory costs (PAR_{comp}). Nevertheless, under more stable conditions (such as in the laboratory) and at longer time scale, it is likely that the gleaner–opportunist tradeoff is mostly driven by the balance between resource allocation to growth machinery (*e.g.* ribosomes) at saturating light (affecting μ_{max}) and allocation to light-harvesting machinery (*e.g.* chlorophyll complexes) at limiting light (affecting α).

Different light acquisition traits will cause big changes in species biovolumes only in the long run. After very few days of new conditions, the now better acclimated species will not necessarily already dominate the group/community. All the dominant species were probably well adapted to the lake conditions prior to our sampling. This could be explained by the assumption of variable conditions in such wind-exposed shallow lake, covering both stagnant and mixing periods.

Effects of constant light intensities gradient

There is a great deal of evidence that interspecific variation in light acquisition traits plays a role in maintaining species diversity through niche partitioning in communities (Litchman and Klausmeier 2001, Schwaderer et al. 2011, Adler et al. 2013). In a stratified eutrophic lake, phytoplankton must cope mostly with spatial heterogeneity in light intensity that declines exponentially with depth. Phytoplankton at the surface receives saturating light, but exclusively on days with little cloud cover. At deeper layers, light availability limits phytoplankton growth. Light availability is also limiting if scums of buoyant colonies/floating macrophytes shade lower depths or colonies self-shade the inner cells. In our study, we mimicked calm thermally stratified conditions by incubating phytoplankton at fixed depths in the lake.

The growth–light relationships of *Anabaena* and *Microcystis* under constant light intersected over the daily PAR gradient. The species displayed different light affinities to limiting and saturating light, thereby exhibiting a gleaner–opportunist tradeoff (Grover 1990). As the gleaner (high α and low PAR_{comp}), *Microcystis* grew more efficiently at limiting light and dominated under constant limiting light. As the opportunist (high μ_{max}), *Anabaena* grew better under saturating light and contributed more to the cyanobacteria biovolume with increasing daily PAR. These alternative light utilization strategies exhibited after only couple of days allowed coexistence of these species on a gradient of constant PAR while avoiding competitive exclusion. Previous studies also identified the importance of the gleaner–opportunist tradeoff for species coexistence along the PAR gradient (Litchman and Klausmeier 2001). Ultimately our results confirmed that opportunist species (high μ_{max}) are more likely to thrive under saturating light, especially when high losses (e.g. by predation) limit self-shading. In contrast, gleaner species (high α, low PAR_{comp}) are more competitive in highly productive/turbid systems when light levels are low.

The gleaner–opportunist tradeoff was not evident amongst the dominant diatom species. While *Cyclotella* had higher μ_{max} and α than *Aulacoseira*, their PAR_{comp} were similar. Meta-analyses of growth–light experiments on marine diatoms species (Edwards et al. 2015) indicate a positive correlation between μ_{max} and α. High values in both maximal growth rates and growth efficiency at limiting light likely evolved by allowing diatoms to survive in turbulent systems where they are usually present and where PAR fluctuates between high and low intensities. This evolutionary hard-wiring in the growth traits is apparently still expressed under constant light conditions in our experiment. Interspecific differences in μ_{max} values between diatoms explained why *Cyclotella* contributed more to the biovolume of diatoms with increasing daily PAR. In contrast, the dominance of *Aulacoseira* at limiting light is not explainable by light traits (lower growth efficiency and similar PAR_{comp}). Traits like affinity for nutrients or vulnerability for grazing were excluded in our experiment but act under natural conditions. There, the unicellular *Cyclotella* should suffer from higher grazing losses than the filamentous *Aulacoseira*. This might explain the higher biomass of *Aulacoseira* than of *Cyclotella* in the inocula, which were assembled from the natural system. Our experiment was likely too short to enable drastic changes in relative species biomass at low light where absolute growth rates of both species were low. In the long run, *Cyclotella* should outcompete *Aulacoseira* at all light intensities if our incubation conditions (replete nutrients, low grazing pressure, no sedimentation) are provided.

Our results confirm generally, that under semi-natural conditions, interspecific variation of light acquisition traits can reduce niche overlap within few days thereby precluding competitive exclusion in a spatially heterogeneous light climate. As a consequence, species diversity within the same phytoplankton group is maintained owing to the PAR gradient occurring in the lake. Nevertheless, such constant light conditions would rarely occur in well-mixed water layers.

Effects of fluctuating light under vertical mixing

Under semi-natural conditions, temporal light fluctuations may result in differences in light acquisition parameters of phytoplankton communities incubated either under constant or fluctuating light (Köhler et al. 2018). However, it is still unknown how the species-specific variation in light acquisition traits may affect the coexistence in situ. Thus, it is critical to estimate light acquisition traits under fluctuating light conditions to explain the development of phytoplankton at vertical mixing.

Under fluctuating light conditions, phytoplankton must cope with light heterogeneity that is both spatial (in the water column) and temporal (in our study, diurnal course of light + 20 min fluctuations). Hence, phytoplankton must be acclimated to both mean level and dynamics of light intensity as they have to cope with the probability of the different light intensities and with the speed of changes. Forecasts of phytoplankton development in situ are uncertain if based on growth–light relationships measured under constant light because mean intensity as well as dynamic of light availability may co-limit growth. Indeed, our results showed that strong intraspecific variation in light acquisition traits under constant and fluctuating light affected competitive outcomes.

As was the case for constant light exposure, the cyanobacteria displayed a gleaner–opportunist tradeoff also under fluctuating light. However, the dominant species switched their strategies and dominance patterns: *Microcystis*, gleaner under constant light became opportunist (high μ_{max}) under fluctuating light while *Anabaena*, opportunist under constant light became a gleaner (low PAR_{comp}) under fluctuating light. This intraspecific variation indicates a strong and fast plasticity of cyanobacteria light acquisition traits, explaining the observed changes in relative biovolumes of dominant species after only two days. The reduction of the minimal light requirements of *Anabaena flos-aquae* under fluctuating light (4 h high:4 h low light) compared to constant light has been hypothesized to be one of the reasons of the increased coexistence potential with another cyanobacteria (the filamentous *Phormidium luridum var.*) in the laboratory by Litchman (2003).

In contrast, light utilization strategies of diatoms were not reversed and the competitive outcomes remained similar. Again, these results indicate the strong adaptation of diatoms to vertical mixing (Reynolds 2006). It is also worth noting that diatoms had overall higher growth rates than cyanobacteria. Nevertheless, because of their relatively small size and high density, diatoms must cope with higher losses by sedimentation and grazing. Therefore, in nature, diatoms may attain a lower biomass than cyanobacteria despite faster gross growth.

With increasing μ_{max}, or higher affinity to saturating light, α of both diatoms and cyanobacteria species increased slightly. Such phenomenon could be explained by photosynthesis enhancement whereby opportunists benefit from intermittent saturating light peaks at the water surface to optimize performance (Marra 1978, Kana and Glibert 1987), but which negatively influences their ability to grow at limiting light levels because of increasing maintenance metabolic cost (Richardson et al. 1983).

Realized light niches over the daily PAR and mixing depth gradients

One of the main challenges in community ecology is to understand how environmental variability shapes the community composition and dynamics in situ (Chesson 2000, Adler et al. 2013). We observed that inter- and intraspecific variation in light acquisition traits toward both mean level and dynamics of light intensity enhanced species coexistence over the PAR gradient. Yet the daily PAR received by phytoplankton in lakes depends, amongst other factors, on the surface irradiance and the mixing depth, the latter being inversely related to the daily PAR.

Diatoms displayed the more straightforward scenario. As mixing specialists, diatoms did not modify their light utilization strategies between constant and fluctuating light regimes. The opportunist *Cyclotella* dominated the diatom biovolume along the whole mixing gradient at saturating light, while *Aulacoseira* did so along the whole mixing gradient at limiting light. Under mixing conditions, the dominance of *Aulacoseira* over *Cyclotella* was favoured by its lower compensation light intensity. Their relative contributions along the gradient of fluctuating light regimes were very distinct after two days (Fig. 3D) and amplified after five days of incubation under both light exposures (Fig. 4B). Thus, no region of similar contribution appeared on the daily PAR × mixing depth gradients. However, these results are not fully transferable to natural conditions. Our incubations avoided losses by sedimentation and largely grazing. Under calm conditions, sedimentation should affect the larger *Aulacoseira* more strongly than the single-celled *Cyclotella*. In contrast, the latter is more vulnerable to grazing.

The niche partitioning between the cyanobacteria species was more complicated. The gleaner *Microcystis* strongly dominated cyanobacteria biovolume under stagnant conditions when light was limiting. Under constant saturating light conditions *Anabaena* was dominant. Both species are buoyant and therefore their permanent occurrence in dim layers of a non-mixed lake is unlikely. Instead, we assume that variation in available light is driven solely by changing cloud cover and light distribution within the colonies.

Unlike the diatoms, the cyanobacteria species had similar relative biomasses across a large range of light intensities (from 2 to 5 E m^{-2} day^{-1}) under both constant and fluctuating light exposure (Fig. 4A). This phenomenon might be, at least partly, explained by self-shading inside of colonies which is poorly understood so far. Nonetheless, it is conceivable that the development of the colonial cyanobacterial opportunist allowed the gleaner to develop because of the limiting effects of self-shading in the colony. On the other hand, at limiting

light levels, only the gleaner with very low light requirements could thrive. This explains the observed higher differences in growth rates and relative biovolumes of species at limiting than at saturating light. Thus, cyanobacteria species may coexist under both stable and mixing conditions at sub-saturating irradiances, and a drastic increase or decrease of the daily PAR may quickly favour the opportunist or gleaner species respectively. Cyanobacteria were affected by vertical mixing with *Anabaena* and *Microcystis* switching light utilization strategies, resulting in a niche partitioning along gradients of daily PAR and mixing depth. The gleaner *Anabaena* benefited from vertical mixing deeper than 0.5 m when the daily PAR was low, and from its higher initial biovolume. *Microcystis* could not outcompete the latter because of its high compensation light intensity under fluctuating light. However, at shallow mixing depths (below 0.5 m deep) a region of similar contribution existed owing to lower interspecific differences in absolute growth rates at saturating than at limiting light.

Our study points to the mechanistic linkages between more natural light environment and phytoplankton dynamics in Lake TaiHu. That said, our goal was not to forecast the development of phytoplankton communities in this particular lake under mixed or stratified conditions. We investigated only one frequency of light fluctuation (20 min) and the light dynamics within the lake itself will be more stochastic, operating at different temporal scales. The observed light-dependency of growth is caused by physiological mechanisms which act at different time scales. However, our experiment resembled natural conditions much better than any approach that neglects light dynamics or species interactions. We advocate approaches that target the variation in light acquisition traits under constant and fluctuating light directly as these may counter predictions made on a species-by-species basis.

Conclusions

High biodiversity of natural phytoplankton communities has been attributed primarily to eco-evolutionary responses of phytoplankton groups to different levels of constant light exposure (i.e. variation across depth only). Our study demonstrates under semi-natural conditions the existence of interspecific variation in light affinities allowing the coexistence of species with different light utilization strategies in spatially heterogeneous light conditions. In addition, the overlooked intraspecific variation in light acquisition traits under fluctuating light impacted the community composition. We demonstrated for the first time that vertical

mixing may alter, or even reverse, light utilization strategies of phytoplankton species. Non-equilibrium conditions increase the amount of niches where acclimated species may thrive, allowing coexistence and avoiding competitive exclusion even in seemingly homogeneous environments.

Acknowledgements - We thank Thomas Mehner and the participants of the workshop 'Scientific Writing' at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries for helpful discussions on an early stage of the manuscript. We acknowledge Katrin Preuß for the species determination, Wenhao Ding for logistic help during our experiment, Tom Shatwell, Kate Laskowski and Ramsy Agha for help with the statistics and Nicolas Guislain for graphic support. Facilities were provided by the Nanjing Institute of Geography and Limnology and Chinese Academy of Sciences. We thank Pr. Qin for offering these facilities.

Funding – This work is part of the Sino-German water supply project SIGN funded by the German Ministry of Education and Research (Grant number 02WCL1336D to AG and JK). BEB acknowledges funding from the Humboldt Foundation for supporting her temporary stay at the IGB in Germany.

Conflicts of interest

The authors declare no conflict of interest.

Supplementary material (available online as Appendix oik-05297 at <www.oikosjournal.org/appendix/oik-05297>). Appendix 1.

General discussion

Few parameters characterize the light dependency of phytoplankton growth: the compensation light intensity $PAR_{comp\mu}$, where production and losses are balanced, the growth efficiency at sub-saturating light α_{μ}, and the maximum growth rate at saturating light μ_{max}. These parameters have been measured in the laboratory for many phytoplankton species at constant irradiances and have been used to explain phytoplankton distribution along environmental light gradients (Schwaderer et al. 2011). However, only very few studies measured growth at a sufficient number of mean light intensities to estimate these parameters under fluctuating light (Nicklisch et al. 2008, Shatwell et al. 2012). Thus, accurate predictions of phytoplankton development under fluctuating light exposure remain difficult to make. This PhD thesis does not intend to directly extrapolate few experimental results to aquatic systems – but rather to improve the mechanistic understanding of the variation of the light-dependency of growth under light fluctuations and effects on phytoplankton development. In the following I discuss the effects of fluctuating light on the three main growth-light parameters, the dependency among parameters and consequences of their variation under fluctuating light for phytoplankton development.

1. Growth efficiency

In Lake TaiHu (Guislain and Köhler, under review) and in the Three Gorges Reservoir TGR (Köhler et al. 2018) we mimicked vertical mixing and induced 20 min period fluctuating light regime by computer-controlled motion of subsamples from phytoplankton communities. Both investigations gave similar results. The response of phytoplankton to fluctuating light

clearly depended on the studied timescale. In the short-term (seconds to minutes), light fluctuations seemed to enhance community ETR-based photosynthesis measured before sunrise. On a daily scale however, efficiencies of daily ETR and daily net oxygen production were lower for phytoplankton incubated under fluctuating compared to under constant light. More, the efficiencies of community growth at sub-saturating light α_μ were significantly lower under fluctuating than under constant light. We thus expected α_μ of mixed phytoplankton to be influenced by different frequency distributions of light and photoaccclimation to fluctuating light.

The effect of different frequency distributions of light intensity was estimated by calculating the daily ETR at a temporal resolution of 75 sec using photosynthesis (ETR)-light parameters and the instantaneous light intensities experienced by vertically moved and fixed samples. By doing so, were covered: the effect of nonlinear photosynthesis-light dependency and effective photoperiod. In Lake TaiHu (Guislain and Köhler, under review), mixing caused declines of 39 % in community growth efficiency α_μ and 13 % in efficiency of daily ETR (1/3 of α_μ). In the Three Gorges Reservoir (Köhler et al. 2018) we observed a reduction in α_μ of 64% and in efficiency of daily ETR of 47% (3/4 of α_μ). Therefore, a large part of the observed declines in α_μ under fluctuating compared to constant light could be explained by the different frequency distributions of light. This estimation may be further improved by taking into account the diurnal variations in electron transport rate. The remaining difference should be attributed to diurnal changes in photosynthesis and light-dependent losses for biosynthesis. The factors of variation of the community growth efficiency under fluctuating light are discussed in the following paragraphs.

1.1. Effects of the frequency distribution of light intensities

Only periods of the day with light intensities above 10 µE $m^{-2}s^{-1}$ (Ryther 1956, Falkowski and Owens 1978, 1980, Langdon 1988) are considered as available for production and define the "effective daylength". The 10 µE $m^{-2}s^{-1}$ threshold has been used in Chapters 1 and 2, and experimentally verified at Lake TaiHu for communities incubated under fluctuating and constant light. Indeed, it is similar to the compensation light intensities Guislain and Köhler (under review) extracted from the 20 min linear net oxygen production-light relations

($PAR_{compP,20}$) on a dim day under fluctuating (8.82 µE $m^{-2}s^{-1}$) and constant light (7.23 µE $m^{-2}s^{-1}$). In both the TGR and Lake TaiHu, mixed algae were always longer exposed to irradiances below 10 µE m^{-2} s^{-1} compared to fixed algae receiving the same daily PAR. This shortens the effective daylength for production of mixed algae and *de facto* explains part of the decline in their α_μ. This is in line with studies showing that reduced effective daylength induced by light fluctuations decreases species-specific growth rates of algae (Nicklisch 1998, Nicklisch and Fietz 2001, Nicklisch et al. 2008, Shatwell et al. 2012). Simulating deeper mixing (or shorter effective daylength) under laboratory conditions led to lower α_μ of single species (Nicklisch and Fietz 2001, Shatwell et al. 2012). These differences increased with declining euphotic to mixing depth ratio.

Nonlinearity of the light-dependency of growth was also assumed to be responsible for the decline in α_μ at vertical mixing (Thornley 1974, Dromgoole 1988, Litchman 2000). The rationale is that saturating light intensities allow for less growth per available photon than under sub-saturating light. Hence, irradiances above the onset of growth saturation $I_{k\mu}$ increase the mean daily light supply but not growth rates. Accordingly, a higher percentage of saturating light received by mixed algae compared to fixed algae should reduce their α_μ at vertical mixing at the same mean sub-saturating light intensity. To be effective on the growth efficiency, this effect of nonlinearity requires high surface irradiance and low optical mixing depth ($\varepsilon * z_{mixing}$) that allows exposure of phytoplankton to growth saturating light intensities. Such conditions were often observed in the TGR but much less in Lake TaiHu. The mean surface PAR (E m^{-2} d^{-1}) was high at the TGR ([2.44-31.23], average = 16.98) and low at Lake TaiHu ([1.36-12.58], average = 4.78). More, the vertical light attenuation (m^{-1}) was low in the TGR ([0.91-1.19]) and high in Lake TaiHu ([5.19-5.22]). To better assess the effect of nonlinearity on growth, Guislain and Köhler (under review) calculated every 75 sec the time proportion spent at irradiances above the onset of saturation $Ik_{P,20}$ for a sunny and a dim day, and not Ik_μ (used by Köhler et al. 2018). They used $Ik_{P,20}$ because the onset of growth saturation Ik_μ is meaningful only at the 24 h timescale and already integrates diurnal changes in photosynthesis and light-dependent losses (Gibson and Foy 1983). Overall, in a light-limited system such as Lake TaiHu, most of samples received limiting light (below $Ik_{P,20}$) even during sunny days. When algae receive such low daily proportion of saturating light, it seems unlikely that nonlinearity plays a prominent role in the decline of the efficiency of net production α_P and growth α_μ under fluctuating light.

1.2. Effects of photoacclimation

The remaining 2/3 and 1/4 of the growth efficiency α_μ gap observed respectively in Lake TaiHu and in the TGR should be caused by light-dependent losses or by imperfect acclimation to fluctuating light. Photoacclimation processes are species and timescale dependent. At the timescale of Langmuir cells, phytoplankton can acclimate to light fluctuations by state-transitions (Falkowski et al. 1994) and changes in the activation state of Rubisco (MacIntyre et al. 2000) for instance. The xanthophyll cycle is another important fast photoacclimation mechanism in diatoms and chlorophytes, but is not possessed by cyanobacteria or cryptophytes (*e.g.* Demming-Adams and Adams 1996). These mechanisms are based on assembly of enzymes/pigments or on dissipation of absorbed energy. Thus, they inevitably reduce the efficiency of conversion of irradiance into biomass compared to constant light of the same mean intensity (*e.g.* Su et al. 2012).

Enhanced losses for biosynthesis (*e.g.* respiration, exudation) may have also contributed in reducing α_μ under fluctuating light. In Lake TaiHu, no difference in night respiration rates after sub-saturating light was noted between constant and fluctuating light. Nonetheless, some studies observed higher rates of respiration (Grande et al. 1989, Luz et al. 2002) and exudation (Zlotnik and Dubinsky 1989, Marañón et al. 2004) in the light compared to the dark. Measuring phytoplankton respiration in semi-natural conditions remains challenging because of the simultaneous oxygen consumption by bacteria and very small grazers. In the laboratory, Guislain and Köhler (in prep.) found that within 20 min light cycles, the net production rates of *Microcystis aeruginosa* exposed to increasing irradiance levels were higher than when exposed to the same intensity but in the decreasing order. Without observed changes in photosynthesis, this rapid hysteresis in net oxygen production rates was mostly due to a fast (minute timescale) light-dependent increase in oxygen consumption by dark respiration. It is very likely that this rapid increase of dark respiration under 20min period fluctuating light eventually decreases α_μ of mixed algae. Overall, our experimental setup provides only a coarse approximation to more complex mixing conditions. In natural environments, the movement of phytoplankton through the water column is certainly less predictable than applied in our experiment (MacIntyre 1993). More irregular movement of algae in the lake may require more flexibility and perhaps faster responses to light fluctuations that may even more hamper the conversion efficiency of light energy into biomass.

2. Maximum growth rates

In both lakes investigated, the maximal growth μ_{max} of algae incubated at fixed depths was slightly lower than of mixed algae, albeit non-significantly different. At high surface irradiance and low optical mixing depth ($\varepsilon^* z_{mixing}$), phytoplankton transported over moderate vertical distances may receive saturating light intensities for growth in the largest part of the mixed water column. If most of the fluctuating light exceeds Ik_{μ} and is not inhibiting, mixed phytoplankton should have similar μ_{max} as algae fixed at an optimum depth (Litchman 2000, Dimier et al. 2009). Guislain and Köhler (under review) and Köhler et al. (2018) observed that the community maximal daily ETR-based photosynthesis and net oxygen production P_{max} were slightly higher under constant than under fluctuating light supply. This could be explained by shorter effective daylength for algae incubated under fluctuating compared to constant light. Here, a contradiction arises. Only different losses for biosynthesis between light regimes may explain higher maximal daily photosynthesis and production of fixed algae while having lower μ_{max} than mixed algae. I discuss the losses at saturating light under constant and fluctuating light supply in the following paragraphs.

2.1. Constant light supply

Guislain and Köhler (under review) analysed the 20 min net production of fixed algae and could explain increasing losses for growth with light by photo-inhibition. During a sunny day at Lake TaiHu, only the samples incubated at the surface and at 20 cm depth were exposed longer than mixed algae to irradiances above $Ik_{P,20}$. For these samples, 20 min net production rates measured at increasing irradiances (in the morning) were lower than when exposed to the same intensity but in the decreasing order (in the afternoon). This hysteresis in net production rates may be explained by diurnal changes in oxygen production and/or consumption. During photo-inhibition, excessive photosynthetic excitation damages the photosystems and requires additional respiratory costs for repair and photo-protection. For instance, enhancement of protein synthesis (*e.g.* for protein D1 of the Photosystem II) by phytoplankton exposed to inhibiting irradiances requires additional respiratory costs (Richardson et al. 1983, Ferris and Christian 1991, Long et al. 1994, Raven 2011, see Box 1). Moreover, photo-inhibition leads inevitably to decreased photosystems activity and

photosynthesis foregone during photorepair (Raven 2011). In both investigations, it is plausible that the effects of inhibition on surface fixed algae were observed even after 12h darkness as showed by lower ETR_{max} and Ik_{ETR} of surface algae compared to deeper fixed samples. Finally, during the night in Lake TaiHu, the significant increase of night respiration rates with growth could have compensated for the additional production observed at constant compared to fluctuating light. This was probably also the case in the TGR.

2.2. Fluctuating light supply

In contrast with fixed algae, Guislain and Köhler (under review) found no growth dependency of the night respiration rates under fluctuating light. Therefore, it is likely that mixed phytoplankton covered their increasing energy demand for biosynthesis and maintenance at faster growth by dark respiration during the day. Yet, no hysteresis in the net oxygen production rates that could have indicated light-dependent increase of dark respiration during the day was observed. However, at the timescale of Langmuir cells, Guislain and Köhler (in prep.) observed in the laboratory that *Microcystis aeruginosa* (isolated from Lake TaiHu) increases its oxygen consumption by dark respiration few minutes only after exposure to increasing light intensities. This result is best explained by the accumulation of carbohydrates during intermittent light peaks (at increasing irradiance) and mobilization of these fresh photosynthetic products by rapid enhancement of dark respiration for maintenance and biosynthesis at decreasing irradiance. Many authors indeed assumed mixed algae to be able to take advantage of cyclic periods in effective darkness for rapidly triggering respiration, relaxing their photosystems and efficiently coupling light and dark reactions during the day, especially in highly turbid systems such as Lake TaiHu (Beardall et al. 1994, Ibelings et al. 1994, Helbling et al. 2013). Longer periods of time spent below 10 $\mu E\ m^{-2}\ s^{-1}$ for mixed algae at Lake TaiHu and TGR should have also been advantageous at daily saturating irradiance (Geider and Osborne, 1989).

Vertically mixed algae may also benefit from short exposure time to surface irradiance. As a matter of fact, the effects of high light exposure on algal photosynthesis and growth are dosage-dependent and the mitigation of inhibition by turbulent mixing has been already demonstrated (Marra 1978, Grobbelaar 1985, Ibelings et al. 1994, Köhler et al. 2001). Mixed algae have been shown to develop efficient strategies to better exploit light peaks occurring at the surface while avoiding photo-inhibition (Ferris and Christian 1991 for review). The

enhancement of potential photosynthesis under fluctuating light has been already reported (Kana and Glibert 1987) and validated for communities experiencing vertical mixing by Köhler et al. (2018) and Guislain and Köhler (under review). Indeed, the average maximal photosynthesis (ETR_{max}) of mixed algae was always significantly higher than of fixed algae.

3. Compensation light intensity for growth

Under nutrient-replete steady-state conditions, phytoplankton grow until self-shading reduces the mean light intensity in the mixed layer to $PAR_{comp\mu}$. Almost all estimates of $PAR_{comp\mu}$ are based on measurements of growth (Hobson and Guest 1983, Falkowski et al. 1985) or photosynthesis and losses (Langdon 1988) under constant light. In stratified water columns, phytoplankton may adapt to relatively constant low light to form distinct deep chlorophyll maxima. Some adaptive strategies involve the reduction of metabolic maintenance costs (*e.g.* lower dark respiration) and increased photosynthetic efficiency (*e.g.* higher absorption cross section, higher ratio of photosynthetic to protective pigments, see Dubinsky and Stambler 2009). These acclimations result in lowered $PAR_{comp\mu}$. Only very few compensation light intensities were experimentally determined under fluctuating light. Köhler et al. (2018) observed that phytoplankton communities needed 3.3 times higher daily PAR to compensate losses under fluctuating light ($PAR_{comp\mu}$ = 2.50 ± 0.30 E m^{-2} d^{-1}) compared to under constant light ($PAR_{comp\mu}$ = 0.76 ± 0.13 E m^{-2} d^{-1}). Their findings are supported by very few relevant field studies showing much higher minimum daily light requirements of phytoplankton under mixing conditions than for algae adapted to constant low light (Riley 1957, Siegel et al. 2002). According to Köhler et al. (2018) this difference in $PAR_{comp\mu}$ is caused by longer periods of the day spent at very low light intensities (<10 µE m^{-2} s^{-1}) by algae incubated at mixed compared to fixed depths. Compensation light intensities for community growth $PAR_{comp\mu}$ at Lake TaiHu were much lower than at the Three Gorges Reservoir. The vertical attenuation coefficient at Lake TaiHu was about 5 times higher and the global radiation was about 3 times lower than at the TGR. Therefore, algae in Lake TaiHu should be adapted to relatively low light supply and may explain their very low $PAR_{comp\mu}$. However, large inter and intra-specific variations in $PAR_{comp\mu}$ of dominant species were observed (Guislain et al. 2019). It is likely that these fast variations in growth-light traits in Lake TaiHu have dampened the variation in $PAR_{comp\mu}$ of communities incubated under mixed compared to under fixed depths (Guislain and Köhler, under review). The studies of Guislain

et al. (2019) and Guislain and Köhler (in prep.) provide valuable insights on the mechanistic understanding of $PAR_{comp\mu}$ under fluctuating light. It is discussed in the following paragraph §4.

The compensation light intensity for growth is crucial for calculations of the critical mixing depth[2]. It is the depth of the surface mixed layer with a mean light intensity approaching $PAR_{comp\mu}$. Therefore, estimates of the critical depth are as precise as $PAR_{comp\mu}$. As was demonstrated in Köhler et al. (2018), the estimation of $PAR_{comp\mu}$ under constant light condition may seriously underestimate the minimum light requirements of phytoplankton in mixed water layers. Accordingly, it overestimates the critical mixing depth. Estimations of the critical depths in turbulent systems become even more complex at the species level (Guislain et al. 2019).

4. Tradeoff between growth-light traits

Species can devote only a limited amount of energy to the acquisition of a particular resource. Therefore, physiological tradeoffs[3] are expected between light acquisition traits, *e.g.* between the maximum growth rate μ_{max} (at saturating light) and growth efficiency α_μ (at limiting light) (Litchman and Klausmeier 2008). μ_{max} and α_μ are influenced by the energy allocated to growth (*e.g.* ribosomes) and light-harvesting machinery (*e.g.* chlorophyll complexes (chlorophyll : C ratio) and accessory pigments) respectively (Langdon 1988, Klausmeier et al. 2004, Litchman 2007, Talmy et al. 2013). Guislain et al. (2019) observed that μ_{max} and $PAR_{comp\mu}$ of dominant species were positively related. They explained this by the enhancement of photosynthesis of species with affinity to high light intensities that increases slightly α_μ but also and to a much larger extent μ_{max} - increasing *de facto* their maintenance respiratory costs and thus $PAR_{comp\mu}$. More precisely, $PAR_{comp\mu}$ is driven by the balance between photosynthesis at limiting light (and thus, light-harvesting machinery) and maintenance respiration (Langdon 1988). Dark respiration consumes oxygen to support

[2]The critical depth z_{crit} is the thickness of the thoroughly mixed water column in which the mean light intensity equals $PAR_{comp\mu}$. Using the Lambert-Beer's law, z_{crit} can be approximated using measured intensities of the surface PAR, the mean vertical light attenuation coefficient and $PAR_{comp\mu}$.

[3]The models used to describe the growth (and production)-light relationships do not include any mathematical tradeoffs between parameters. The three parameters are independent and bring separate information to the fit of measurements.

biosynthesis and cell growth (Ferris and Christian, 1991). Therefore, the maintenance respiration to growth ratio should be higher for high-light acclimated, fast-growing species with high μ_{max} (but high $PAR_{comp\mu}$ at limiting light) than for low-light acclimated species (high α_{μ} and low $PAR_{comp\mu}$). In addition, photo-damages at inhibiting irradiance require additional respiratory costs (Richardson et al. 1983) that may also increase the $PAR_{comp\mu}$ of species.

A similar tradeoff between low *vs.* high light has been observed by Guislain and Köhler (in prep.) at a much higher time resolution (order of a minute). They showed that within laboratory simulated Langmuir cells of 20 min period, maximal net oxygen production P_{max} at increasing light levels resulted in an increase of PAR_{compP} at decreasing light levels of the same intensity. Within these 20 min light cycles, no change in electron transport rate was observed between light supplied in the increasing and in the decreasing order. This indicated that the variations in PAR_{compP} was only due to dark respiratory costs and depended on P_{max}. The photosynthetic products (*i.e.* carbohydrates) built at saturating light may be thus directly mobilized by rapid enhancement of dark respiration at decreasing light intensities. To our knowledge, this is the very first time that this tradeoff is observed at a single minute resolution under fluctuating light. To go further, it is very likely that a more high time resolution analysis of the carbohydrates content would bring more insights on the physiological basis of this tradeoff.

5. Impact of fluctuating light on the non-equilibrium coexistence

One of the main challenges in community ecology is to understand how environmental variability shapes the community composition and dynamics *in situ* (Chesson 2000, Adler et al. 2013). There is a great deal of evidence that interspecific variation in light acquisition traits plays a role in maintaining species diversity through niche partitioning in communities (Litchman and Klausmeier 2001, Schwaderer et al. 2011, Adler et al. 2013). However, it is still unknown how species light acquisition traits variation under fluctuating light may alter niche partitioning and thus species coexistence in bulk phytoplankton communities. Guislain et al. (2019) used the light acquisition traits of dominant species in order to explain relative biomasses along the daily PAR gradient under both constant and fluctuating light (see Box 2). Under both light regimes, high μ_{max} of species described the competitive dominance at

saturating light. In contrast, species with low $PAR_{comp\mu}$ were more efficient at limiting light and almost always dominated their group biovolume under such condition. This study demonstrated that $PAR_{comp\mu}$ was more relevant than α_{μ} to determine the ability of a species to outcompete others under constant and fluctuating limiting light supply.

Because of the "high *vs.* low light" tradeoff (see General Discussion §4), species had affinities either to limiting or to saturating light - thereby exhibiting a gleaner-opportunist tradeoff (Grover 1990). These alternative light utilization strategies of species (inter-specific variation in light acquisition traits) allowed the coexistence of different species on a gradient of constant PAR while avoiding competitive exclusion. Such conditions are typically found in stratified lakes where phytoplankton must cope with exponential decline of light intensity with depth. More interestingly, Guislain et al. (2019) demonstrated for the first time that vertical mixing may alter, or even reverse, light utilization strategies of phytoplankton species within couple of days. The intra-specific variation in light acquisition traits under fluctuating light increased the niche space for acclimated species, precluding competitive exclusion. Overall, fluctuating light clearly increase the amount of niches where acclimated species may thrive, allowing coexistence and avoiding competitive exclusion even in seemingly homogeneous environments.

Box 2

In classical Biodiversity and Ecosystem Functioning experiments, monocultures are generally used to measure productivity or standing biomass. Polycultures are then used to study positive or negative overyielding, in comparison to monocultures. In the semi-natural conditions investigation performed in Lake TaiHu (China), demographic traits of dominant species are estimated directly in the polyculture (where species may interact), not independently in monocultures (where species do not interact). Hence, the way species traits are measured might integrate interspecific competition. In that case, species traits depend on the community context. We deliberately measured light acquisition traits in the community to better understand how variation in the eco-physiology of species could enhance their non-equilibrium coexistence. Lawrence et al. (2012) found that species diverged more in resource use in polycultures compared to species cultured separately, proving the character displacement of interactive species to reduce resource overlap. Assessing the variation in light acquisition traits and species biovolumes directly in the community should thus be more meaningful and closer to the natural conditions than in monoculture experiments. Moreover, the investigated phytoplankton community was well-adapted to conditions in Lake TaiHu. In such shallow, wind-exposed lakes, calm periods or mixing events rarely last longer than few days. We tried to avoid other controlling factors (nutrient shortage, temperature changes, grazing) in our incubations. Under such conditions and after only few days of treatment, phytoplankton dynamics should be mostly driven by rapid acclimation to changes in light climate and not by interspecific competition.

Another point should be addressed. In Guislain et al. (2019), competitive exclusion or coexistence is explained on the base of growth rates measured in the very same context. Here, we do not use the measured growth-light relationships as predictors of potential species performances (“fundamental light niches”) in a community along the light gradient. We rather used the growth-light relationships as descriptors of “realized light niches” to further understand how variation in species light acquisition traits could influence the species coexistence in a lake community under stratified or mixed conditions.

Conclusion

The present PhD thesis provides evidence for substantial effects of fluctuating light on the eco-physiology of phytoplankton. Both experiments performed under semi-natural conditions in Lake TaiHu and at the Three Gorges Reservoir gave similar results. The significant decline in community growth efficiencies α_μ under fluctuating light was caused for a great share by different frequency distribution of light intensities that shortened the effective daylength for production. The remaining gap in community α_μ was attributed to species-specific photoacclimation mechanisms and to light-dependent respiratory losses. In contrast, community maximal growth rates μ_{max} were similar between incubations at constant and fluctuating light. At daily growth saturating light supply, differences in losses for biosynthesis between the two light regimes were observed. Phytoplankton experiencing constant light supply were saturated at lower light intensities than mixed algae. Moreover, communities incubated at the surface were photo-inhibited. Photo-inhibition led to photosynthesis foregone and additional respiratory costs for photosystems repair. On the contrary, intermittent exposure to low and high light intensities prevented photo-inhibition of mixed algae but forced them to develop alternative light strategy. Compared to fixed algae, they better harvest and exploit surface irradiance by enhancing their photosynthesis. By simulating 20 min Langmuir cells in the laboratory, we showed that *Microcystis aeruginosa* increased its oxygen consumption by dark respiration in the light few minutes only after exposure to increasing light intensities. More, we proved that within a simulated Langmuir cell, the net production at saturating light and the compensation light intensity for production at limiting light are positively related. These results are best explained by an accumulation of photosynthetic products at increasing irradiance and mobilization of these fresh resources by rapid enhancement of dark respiration for maintenance and biosynthesis

at decreasing irradiance. To go further, analysing at high time resolution the dynamics of carbohydrates content could bring more insights on the physiological basis of this tradeoff. At the daily timescale, we showed that the enhancement of photosynthesis at high irradiance for biosynthesis of species increased their maintenance respiratory costs at limiting light. Species-specific growth at saturating light μ_{max} and compensation light intensity for growth $PAR_{comp\mu}$ of species incubated in Lake TaiHu were positively related. Because of this species-specific physiological tradeoff, species displayed different light affinities to limiting and saturating light - thereby exhibiting a gleaner-opportunist tradeoff. In Lake TaiHu, we showed that inter-specific differences in light acquisition traits (μ_{max} and $PAR_{comp\mu}$) allowed coexistence of species on a gradient of constant light while avoiding competitive exclusion. More interestingly we demonstrated for the first time that vertical mixing (inducing fluctuating light supply for phytoplankton) may alter or even reverse the light utilization strategies of species within couple of days. The intra-specific variation in traits under fluctuating light increased the niche space for acclimated species, precluding competitive exclusion.

The eco-physiology of phytoplankton under fluctuating light requires more simultaneous studies of physiology and turbulence-driven vertical movement of planktonic algae. Overall, this PhD thesis contributes to a better understanding of phytoplankton eco-physiology under fluctuating light supply and potentially to more reliable predictions of phytoplankton development under certain weather conditions or climate change scenarios.

References

Abu-Ghosh, S., Fixler, D., Dubinsky, Z., Solovchenko, A., Zigman, M., Yehoshua, Y., et al. (2015). Flashing light enhancement of photosynthesis and growth occurs when photochemistry and photoprotection are balanced in *Dunaliella salina*. *Eur. J. Phycol.* 50, 469–480. doi:10.1080/09670262.2015.1069404.

Adler, P. B. et al. 2013. Trait-based tests of coexistence mechanisms. - Ecol. Lett. 16: 1294–1306.

Akaike, H. (1974). A new look at the statistical model identification. - IEEE transactions on automatic control 19: 716-723.

Amarasekare, P. 2003. Competitive coexistence in spatially structured environments: A synthesis. - Ecol. Lett. 6: 1109–1122.

Anderson, J. M., Y. I. Park, and W. S. Chow. 1997. Photoinactivation and photoprotection of photosystem II in nature. Physiol. Plant. 100: 214–223. doi:10.1111/j.1399-3054.1997.tb04777.x

Avendano-Coletta, D. and Schubert, H. 2005. Oxygen evolution and respiration of the cyanobacterium *Synechocystis sp* PCC 6803 under two different light regimes applying light/dark intervals in the time scale of minutes. - Physiol Plant 125: 381–391.

Barbieri, E. S., V. E. Villafane, and E. W. Helbling. 2002. Experimental assessment of UV effects on temperate marine phytoplankton when exposed to variable radiation regimes. Limnol. Oceanogr. 47: 1648–1655. doi:10.4319/ lo.2002.47.6.1648

Bates, D. M., and Watts, D. G. (1988). *Nonlinear Regression Analysis and its applications*. Doi:10.2307/2289810.

Beardall, J. (1989). Photosynthesis and photorespiration in marine phytoplankton. *Aquatic Botany*, *34*(1-3), 105-130.

Beardall, J., Burger-wiersma, T., Rijkeboer, M., Sukenik, A., Lemoalle, J., Dubinsky, Z., et al. (1994). Studies on enhanced post-illumination respiration in microalgae. *J. Plankton Res.* 16, 1401–1410. doi:10.1093/plankt/16.10.1401.

Beier, C., Beierkuhnlein, C., Wohlgemuth, T., Penuelas, J., Emmett, B., Körner, C., et al. (2012). Precipitation manipulation experiments - challenges and recommendations for the future. *Ecol. Lett.* 15, 899–911. doi:10.1111/j.1461-0248.2012.01793.x.

Beisner, B. E. 2001. Plankton community structure in fluctuating environments and the role of productivity. - Oikos 95: 496–510.

Bidigare, R. R., Ondrusek, M. E., Morrow, J. H., & Kiefer, D. A. (1990). In-vivo absorption properties of algal pigments. In *Ocean Optics X* (Vol. 1302, pp. 290-302). International Society for Optics and Photonics.

Boelen, P., van de Poll, W. H., van der Strate, H. J., Neven, I. A., Beardall, J., and Buma, A. G. J. (2011). Neither elevated nor reduced CO_2 affects the photophysiological performance of the marine Antarctic diatom *Chaetoceros brevis*. *J. Exp. Mar. Bio. Ecol.* 406, 38–45. doi:10.1016/j.jembe.2011.06.012.

Bright, D. I., and A. E. Walsby. 2000. The daily integral of growth by *Planktothrix rubescens* calculated from growth rate in culture and irradiance in Lake Zürich. New Phytol. 146: 301–316. doi:10.1046/j.1469-8137.2000.00640.x

Brunet, Christophe, and Johann Lavaud. "Can the xanthophyll cycle help extract the essence of the microalgal functional response to a variable light environment?." *Journal of Plankton Research* 32.12 (2010): 1609-1617.

Chesson, P.L. and Case, T.J. 1986. Overview: nonequilibrium community theories: chance, variability, history, and coexistence. - Community Ecology: 229-239 (ed. J. Diamond and T.J. Case). Harper and Row, New York.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. - Annual Review of Ecology and Systematics 31: 343-366.

Cianelli, D., M. R. D'Alcala, V. Saggiomo, and E. Zambianchi. 2004. Coupling mixing and photophysiological response of Antarctic plankton: A Lagrangian approach. Antarct. Sci. 16: 133–142. doi:10.1017/ S0954102004001968

Cosper, E. (1982). Influence of light intensity on diel variations in rates of growth, respiration and organic release of a marine diatom: comparison of diurnally constant and fluctuating light. *J. Plankton Res.* 4, 705–724. doi:10.1093/plankt/4.3.705.

Cottingham, K. L., Lennon, J. T., Brown, B. L., Cottingham, K. L., Lennon, J. T., and Brown, B. L. (2005). Knowing when to draw the line : designing more informative ecological exper. *Front. Ecol. Environ.* 3, 145–152. doi:10.2307/3868542.

Cullen, J. J., and Lewis, M. R. (1988). The kinetics of algal photoadaptation in the context of vertical mixing. *J. Plankton Res.* 10, 1039–1063. doi:10.1093/plankt/10.5.1039.

Cullen, J. J., Neale, P. J., & Lesser, M. P. (1992). Biological weighting function for the inhibition of phytoplankton photosynthesis by ultraviolet radiation. *Science*, *258*(5082), 646-650.

Cullen, J. J., and MacIntyre, J. (1998). Behavior , physiology and the niche of marine phytoplankton. *Nato Asi Ser. G Ecoogical Sci.* 41, 559–580.

De Boeck, H. J., Vicca, S., Roy, J., Nijs, I., Milcu, A., Kreyling, J., et al. (2015). Global Change Experiments: Challenges and Opportunities. *Bioscience* 65, 922–931. doi:10.1093/biosci/biv099.

Demmig-Adams, B., and W. W. Adams. 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends Plant Sci. 1: 21–26. doi:10.1016/S1360-1385(96)80019-7

Denman, K. L., and Gargett, A. E. (1983). Time and space scales of vertical mixing and advection of phytoplankton in the upper ocean. *Limnol. Oceanogr.* 28, 801–815. doi:10.4319/lo.1983.28.5.0801.

Dimier, C., Brunet, C., Geider, R., and Raven, J. (2009). Growth and photoregulation dynamics of the picoeukaryote *Pelagomonas calceolata* in fluctuating light. *Limnol. Oceanogr.* 54, 823–836. doi:10.4319/lo.2009.54.3.0823.

Dromgoole, F. I. (1988). Light Fluctuations and the Photosynthesis of Marine Algae. II. Photosynthetic Response to Frequency, Phase Ratio and Amplitude. *Funct. Ecol.* 2, 211. doi:10.2307/2389697.

Duan, H., Ma, R., Xu, X., Kong, F., Zhang, S., Kong, W., et al. (2009). Two-decade reconstruction of algal blooms in China's Lake Taihu. *Environ. Sci. Technol.* 43, 3522–3528. doi:10.1021/es8031852.

Dubinsky, Z., and Stambler, N. (2009). Photoacclimation processes in phytoplankton: mechanisms, consequences, and applications. *Aquat. Microb. Ecol.* 56, 163–176. doi:10.3354/ame01345.

Edwards, K. F. et al. 2013a. Functional traits explain phytoplankton community structure and seasonal dynamics in a marine ecosystem. - Ecol. Lett. 16: 56–63.

Edwards, K. F. et al. 2013b. Functional traits explain phytoplankton responses to environmental gradients across lakes of the United States. - Ecology 94: 1626–1635.

Edwards, K. F. et al. 2015. Light and growth in marine phytoplankton: allometric, taxonomic, and environmental variation. - Limnol. Oceanogr. 60: 540-552.

Edwards, K. F., Thomas, M. K., Klausmeier, C. A., and Litchman, E. (2016). Phytoplankton growth and the interaction of light and temperature: A synthesis at the species and community level. *Limnol. Oceanogr.* 61, 1232–1244. doi:10.1002/lno.10282.

Falkowski, P. G., and Owens, T. G. (1978). Effects of light intensity on photosynthesis and dark respiration in six species of marine phytoplankton. *Mar. Biol.* 45, 289–295. doi:10.1007/BF00391815.

Falkowski, P. G., and Owens, T. G. (1980). Light- Shade Adaptation. *Plant Physiol.* 66, 592–595. doi:0032-0889/80/66/0592/04.

Falkowski, P. G. (1984). Physiological responses of phytoplankton to natural light regimes. *J. Plankt. Resarch* 6, 295–307. doi:10.1093/plankt/6.2.295.

Falkowski, P. G., Z. Dubinsky, and K. Wyman. 1985. Growth-irradiance relationships in phytoplankton. Limnol. Oceanogr. 30: 311–321. doi:10.4319/lo.1985.30.2.0311

Falkowski, Paul G., and Julie LaRoche. "Acclimation to spectral irradiance in algae." *Journal of Phycology* 27.1 (1991): 8-14.

Falkowski, P. G., Greene, R., and Kolber, Z. (1993). Light utilization and photoinhibition of photosynthesis in marine phytoplankton.

Falkowski, P. G., R. Greene, and Z. Kolber. 1994. Light utilization and photoinhibition of photosynthesis in marine phytoplankton, p. 407–432. In N. R. Baker and J. R. Bowyer [eds.], Photoinhibition of photosynthesis: From molecular mechanisms to the field. Bios Scientific Publishers.

Falkowski, P. G., Barber, R. T., and Smetacek, V. (1998). Biogeochemical controls and feedbacks on ocean primary production. *Science (80-.).* 281, 200–206. doi:10.1126/science.281.5374.200.

Falkowski, P., & Raven, J. (2007). *Aquatic Photosynthesis: (Second Edition)*. Princeton University Press.

Falkowski, P. (2012). The power of plankton. *Nature*, *483*(Suppl 7387), S17.

Ferris, J. M., and Christian, R. (1991). Aquatic primary production in relation to microalgal responses to changing light: A review. *Aquat. Sci.* 53, 187–217. doi:10.1007/BF00877059.

Field, C. B., Behrenfeld, M. J., Randerson, J. T., Field, C. B., Behrenfeld, M. J., and Randerson, J. T. (1998). Primary Production of the Biosphere : Integrating Terrestrial and Oceanic Components Published by : American Association for the Advancement of Science Stable URL : http://www.jstor.org/stable/2896014 Linked references are available on JSTOR for this arti. *Science (80-.).* 281, 237–240.

Fietz, S., and Nicklisch, A. (2002). Acclimation of the diatom *Stephanodiscus neoastraea* and the cyanobacterium Planktothrix agardhii to simulated natural light fluctuations. *Photosynth. Res.* 72, 95–106. doi:10.1023/A:1016052726149.

Flameling, I. a., and Kromkamp, J. (1998). Light dependence of quantum yields for PSII charge separation and oxygen evolution in eucaryotic algae. *Limnol. Oceanogr.* 43, 284–297. doi:10.4319/lo.1998.43.2.0284.

Flöder, S. et al. 2002. The influence of fluctuating light intensities on species composition and diversity of natural phytoplankton communities. - Oecologia 133: 395–401.

Franks, P. J. S. 2015. Has Sverdrup's critical depth hypothesis been tested? Mixed layers vs. turbulent layers. ICES J. Mar. Sci. 72: 1897–1907. doi:10.1093/icesjms/fsu175

Gargett, A. E., and J. R. Wells. 2007. Langmuir turbulence in shallow water. Part 1. Observations. J. Fluid Mech. 576: 27–61. doi:10.1017/S0022112006004575

Geider, R. J., B. A. Osborne, and J. A. Raven. 1985. Light dependence of growth and photosynthesis in *Phaeodactylum tricornutum* (Bacillariophyceae). J. Phycol. 21: 609–619. doi:10.1111/j.0022-3646.1985.00609.x

Geider, R. J., and Osborne, B. a. (1989). Respiration and microalgal growth: a review of the quantitative relationship between dark respiration and growth. *New Phytol.* 112, 327–341. doi:10.1111/j.1469-8137.1989.tb00321.x.

Geider, R. J., MacIntyre, H. L., and Kana, T. M. (1996). A dynamic model of photoadaptation in phytoplankton. *Limnol. Oceanogr.* 41, 1–15. doi:10.4319/lo.1996.41.1.0001.

Gibson, C. E., and Foy, R. H. (1983). The photosynthesis and growth efficiency of a planktonic blue-green alga, Oscillatoria redekei. *Br. Phycol. J.* 18, 39–45. doi:10.1080/00071618300650051.

Gibson, C. E. 1985. Growth rate, maintenance energy and pigmentation of planktonic cyanophyta during one-hour light: Dark cycles. Br. Phycol. J. 20: 155–161. doi:10.1080/00071618500650161

Glover, H. E., Keller, M. D., and Spinrad, R. W. (1987). The effects of light quality and intensity on photosynthesis and growth of marine eukaryotic and prokaryotic phytoplankton clones. *J. Exp. Mar. Bio. Ecol.* 105, 137–159.

Graff, J. R., Westberry, T. K., Milligan, A. J., Brown, M. B., Dall'Olmo, G., Reifel, K. M., et al. (2016). Photoacclimation of natural phytoplankton communities. *Mar. Ecol. Prog. Ser.* 542, 51–62. doi:10.3354/meps11539.

Grande, K. D., Marra, J., Langdon, C., Heinemann, K., and Bender, M. L. (1989). Rates of respiration in the light measured in marine phytoplankton using an 18O isotope-labelling technique. *J. Exp. Mar. Bio. Ecol.* 129, 95–120. doi:10.1016/0022-0981(89)90050-6.

Gregory, T. R. (2001). Coincidence, coevolution, or causation? DNA content, cell size, and the C - value enigma. *Biol. Rev.* 76, 65–101.

Grobbelaar, J. U. (1985). Phytoplankton productivity in turbid waters. *J. Plankton Res.* 7, 653–663. doi:10.1093/plankt/7.5.653.

Grover, J.P. 1990. Resource Competition in a Variable Environment : Phytoplankton Growing According to Monod ' s. - Am. Nat. 136: 771–789.

Guislain, A. et al. 2018. Data from: variation in species light acquisition traits under fluctuating light regimes: implications for non-equilibrium coexistence. – Dryad Digital Repository, <http://dx.doi.org/10.5061/dryad.2rh61qk>.

Guislain, A., Beisner, B. E., and Köhler, J. (2019). Variation in species light acquisition traits under fluctuating light regimes: implications for non-equilibrium coexistence. *Oikos* 128, 716–728. doi:10.1111/oik.05297.

Hancke, K., T. Dalsgaard, M. K. Sejr, S. Markager, and R. N. Glud. 2015. Phytoplankton productivity in an Arctic Fjord (West Greenland): Estimating electron requirements for carbon fixation and oxygen production. PLoS One 10: e0133275. doi:10.1371/journal.pone.0133275

Harris, G. P., and B. B. Piccinin. 1977. Photosynthesis by natural phytoplankton populations. Arch. Hydrobiol. 80: 405–457. doi:10.1016/0198-0149(82)90061-9

Havelková-Doušová, H., Prášil, O., and Behrenfeld, M. J. (2004). Photoacclimation of *Dunaliella tertiolecta* (Chlorophyceae) under fluctuating irradiance. *Photosynthetica* 42, 273–281. doi:10.1023/B:PHOT.0000040600.04502.32.

Helbling, E. W., Carrillo, P., Medina-Sánchez, J. M., Durán, C., Herrera, G., Villar-Argaiz, M., et al. (2013). Interactive effects of vertical mixing, nutrients and ultraviolet radiation: In situ photosynthetic responses of phytoplankton from high mountain lakes in Southern Europe. *Biogeosciences* 10, 1037–1050. doi:10.5194/bg-10-1037-2013.

Hobson, L. A., and K. P. Guest. 1983. Values of net compensation irradiation and their dependence on photosynthetic efficiency and respiration in marine unicellular algae. Mar. Biol. 74: 1–7. doi:10.1007/BF00394268

Hoppe, C. J. M., Holtz, L.-M., Trimborn, S., and Rost, B. (2015). Ocean acidification decreases the light-use efficiency in an Antarctic diatom under dynamic but not constant light. *New Phytol.* 207, 159–71. doi:10.1111/nph.13334.

Hotchkiss, E. R., and Hall, R. O. (2014). High rates of daytime respiration in three streams: Use of $\delta^{18}O_{O2}$ and O_2 to model diel ecosystem metabolism. *Limnol. Oceanogr.* 59, 798–810. doi:10.4319/lo.2014.59.3.0798.

Huisman, J., P. van Oostveen, and F. J. Weissing. 1999. Critical depth and critical turbulence: Two different mechanisms for the development of phytoplankton blooms. Limnol. Ocean- ogr. 44: 1781–1787. doi:10.4319/lo.1999.44.7.1781

Hutchinson, G.E. 1961. The paradox of the plankton. - Am. Nat. 95: 137-145.

Ibelings, B. W., Kroon, B. M. a., and Mur, L. R. (1994). Acclimation of photosystem II in a cyanobacterium and a eukaryotic green alga to high and fluctuating photosynthetic photon flux densities, simulating light regimes induced by mixing in lakes. *New Phytol.* 128, 407–424.

Iluz, D., Alexandrovich, I., and Dubinsky, Z. (2012). The enhancement of photosynthesis by fluctuating light. *Artif. Photosynth.*, 115–134.

Jitts, H. R., C. D. Mcallister, K. Stephens, and J. D. H. Strickland. 1964. The cell division rates of some marine phytoplankters as a function of light and temperature. J. Fish. Res. Board Can. 21: 139–157. doi:10.1139/f64-012

Kana, T. M., and Glibert, P. M. (1987). Effect of irradiances up to 2000 µE m^{-2} s^{-1} on marine *Synechococcus* WH7803-I. Growth, pigmentation, and cell composition. *Deep Sea Res. Part A. Oceanogr. Res. Pap.* 34, 497–516.

Kana, T. M. (1993). Rapid oxygen cycling in *Trichodesmium thiebautii*. *Limnol. Oceanogr.* 38, 18–24. doi:10.4319/lo.1993.38.1.0018.

Kardinaal, W. E. A., Tonk, L., Janse, I., Hol, S., Slot, P., Huisman, J., et al. (2007). Competition for light between toxic and nontoxic strains of the harmful cyanobacterium *Microcystis*. *Appl. Environ. Microbiol.* 73, 2939–2946. doi:10.1128/AEM.02892-06.

Kirilovsky, D., and C. A. Kerfeld. 2016. Cyanobacterial photoprotection by the orange carotenoid protein. Nat. Plants 2: 16180. doi:10.1038/nplants.2016.180

Kirk, J. T. O. (1994). Light and photosynthesis in aquatic ecosystems. Cambridge Univ. Press.

Klausmeier, C. A. et al. 2004. Optimal nitrogen-to-phosphorous stoichiometry of phytoplankton. - Nature 429: 171–174.

Köhler, J. 1997. Measurement of in situ growth rates of phytoplankton under conditions of simulated turbulence. J. Plankton Res. 19: 849–862. doi:10.1093/plankt/19.7.849

Köhler, J., Schmitt, M., Krumbeck, H., Kapfer, M., Litchman, E., and Neale, P. J. (2001). Effects of UV on carbon assimilation of phytoplankton in a mixed water column. *Aquat. Sci.* 63, 294–309. doi:10.1007/PL00001356.

Köhler, J., Wang, L., Guislain, A., and Shatwell, T. (2018). Influence of vertical mixing on light-dependency of phytoplankton growth. *Limnol. Oceanogr.* 63, 1156–1167. doi:10.1002/lno.10761.

Koussoroplis, A. M. et al. 2017. Understanding and predicting physiological performance of organisms in fluctuating and multifactorial environments. - Ecol. Monogr. 87: 178–197.

Langdon, C. (1988). On the causes of interspecific differences in the growth-irradiance relationship for phytoplankton. II. A general review. *J. Plankton Res.* 10, 1291–1312. doi:10.1093/plankt/10.6.1291.

Langmuir, I. (1938). Surface motion of water induced by wind.*Science*, *87*(2250), 119-123.

Lavaud, Johann, Robert F. Strzepek, and Peter G. Kroth. "Photoprotection capacity differs among diatoms: possible consequences on the spatial distribution of diatoms related to fluctuations in the underwater light climate." *Limnology and Oceanography* 52.3 (2007): 1188-1194.

Lawrence, D. et al. 2012. Species interactions alter evolutionary responses to a novel environment. - PLoS Biol. 10.5: e1001330.

Lin, H., Kuzminov, F. I., Park, J., Lee, S., Falkowski, P. G., & Gorbunov, M. Y. (2016). The fate of photons absorbed by phytoplankton in the global ocean. *Science*, *351*(6270), 264-267.

Litchman, E. 1998. Population and community responses of phytoplankton to fluctuating light. - Oecologia 117: 247–257.

Litchman, E. (2000). Growth rates of phytoplankton under fluctuating light. *Freshw. Biol.* 44, 223–235. doi:10.1046/j.1365-2427.2000.00559.x.

Litchman, E. and Klausmeier, C. A. 2001. Competition of phytoplankton under fluctuating light. - Am. Nat. 157: 170–187.

Litchman, E. 2003. Competition and coexistence of phytoplankton under fluctuating light: Experiments with two cyanobacteria. - Aquat. Microb. Ecol. 31: 241–248.

Litchman, E., Klausmeier, C. a., and Bossard, P. (2004). Phytoplankton nutrient competition under dynamic light regimes. *Limnol. Oceanogr.* 49, 1457–1462. doi:10.4319/lo.2004.49.4_part_2.1457.

Litchman, E. 2007. Resource Competition and the Ecological Success of Phytoplankton. - Evol. Prim. Prod. Sea: 351–375.

Litchman, E. and Klausmeier, C. A. 2008. Trait-based community ecology of phytoplankton. - Annu. Rev. Ecol. Evol. Syst. 39: 615–639.

Litchman, E. et al. 2012. Phytoplankton niches, traits and eco-evolutionary responses to global environmental change. - Mar. Ecol. Prog. Ser. 470: 235–248.

Litchman, E., de Tezanos Pinto, P., Edwards, K. F., Klausmeier, C. A., Kremer, C. T., and Thomas, M. K. (2015). Global biogeochemical impacts of phytoplankton: A trait-based perspective. *J. Ecol.* 103, 1384–1396. doi:10.1111/1365-2745.12438.

Liu, L., D. Liu, D. M. Johnson, Z. Yi, and Y. Huang. 2012. Effects of vertical mixing on phytoplankton blooms in Xiangxi Bay of Three Gorges Reservoir: Implications for management. Water Res. 46: 2121–2130. doi:10.1016/ j.watres.2012.01.029

Long, S. P., Humphries, S., and Falkowski, P. G. (1994). Photoinhibition of photosynthesis in nature. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 45, 633–662. doi:10.1146/annurev.pp.45.060194.003221

Longhi, M. L., and Beisner, B. E. (2009). Environmental factors controlling the vertical distribution of phytoplankton in lakes\n10.1093/plankt/fbp065. *J. Plankt. Res.* 31, 1195–1207. doi:10.1093/plankt/fbp065.

Lund, J. W. G. et al. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. - Hydrobiologia 11: 143–170.

Luz, B., Barkan, E., Sagi, Y., & Yacobi, Y. Z. (2002). Evaluation of community respiratory mechanisms with oxygen isotopes: A case study in Lake Kinneret. *Limnol. Oceanogr.* 47, 33–42.

MacIntyre, H. L., Kana, T. M., and Geider, R. J. (2000). The effect of water motion on short-term rates of photosynthesis by marine phytoplankton. *Trends Plant Sci.* 5, 12–17. doi:10.1016/S1360-1385(99)01504-6.

MacIntyre, H. L., Kana, T. M., Anning, T., and Geider, R. J. (2002). Photoacclimation of Photosynthesis Irradiance Response Curves and Photosynthetic Pigments in Microalgae and Cyanobacteria. *J. Phycol.* 38, 17–38. doi:10.1046/j.1529-8817.2002.00094.x.

MacIntyre, S. (1993). Vertical mixing in a shallow, eutrophic lake: Possible consequences for the light climate of phytoplankton. *Limnol. Oceanogr.* 38, 798–817. doi:10.4319/lo.1993.38.4.0798.

Marañón, E., Cermeño, P., Fernández, E., Rodríguez, J., and Zabala, L. (2004). Significance and mechanisms of photosynthetic production of dissolved organic carbon in a coastal eutrophic ecosystem. *Limnol. Oceanogr.* 49, 1652–1666. doi:10.4319/lo.2004.49.5.1652.

Marra, J. (1978). Phytoplankton photosynthetic response to vertical movement in a mixed layer. *Mar. Biol.* 46, 203–208. doi:10.1007/BF00390681.

Marra, J. F., V. P. Lance, R. D. Vaillancourt, and B. R. Hargreaves. 2014. Resolving the ocean's euphotic zone. Deep-Sea Res. Part I Oceanogr. Res. Pap. 83: 45–50. doi: 10.1016/j.dsr.2013.09.005

Masojidek, J., Grobbelaar, J. U., Pechar, L., and Koblížek, M. (2001). Photosystem II electron transport rates and oxygen production in natural waterblooms of freshwater cyanobacteria during a diel cycle. *J. Plankton Res.* 23, 57–66. doi:10.1093/plankt/23.1.57.

Mitrovic, S. M., C. G. Howden, L. C. Bowling, and R. T. Buckney. 2003. Unusual allometry between in situ growth of freshwater phytoplankton under static and fluctuating light environments: Possible implications for dominance. J. Plankton Res. 25: 517–526. doi:10.1093/plankt/25.5.517

Mikko, Tikkanen, et al. "State transitions revisited - a buffering system for dynamic low light acclimation of Arabidopsis." *Plant molecular biology* 62.4-5 (2006): 779.

Müller, P., Li, X. P., & Niyogi, K. K. (2001). Non-photochemical quenching. A response to excess light energy. *Plant physiology*,*125*(4), 1558-1566.

Neale, P. J., Davis, R. F., and Cullen, J. J. (1998). Interactive ozone depletion and vertical mixing on photosynthesis of antarctic phytoplankton. *Nature* 392, 585–589. doi:10.1038/33374.

Nelson, D. M., and W. O. Smith. 1991. Sverdrup revisited: Critical depths, maximum chlorophyll levels, and the control of Southern Ocean productivity by the irradiance-mixing regime. Limnol. Oceanogr. 36: 1650–1661. doi: 10.4319/lo.1991.36.8.1650

Nicklisch, A. (1998). Growth and light absorption of some planktonic cyanobacteria, diatoms and Chlorophyceae under simulated natural light fluctuations. *J. Plankton Res.* 20, 105–119. doi:10.1093/plankt/20.1.105.

Nicklisch, A. and Fietz, S. (2001). The influence of light fluctuations on growth and photosynthesis of *Stephanodiscus neoastraea* (diatom) and *Planktothrix agardhii* (cyanobacterium). *Archiv fur Hydrobiologie*, *151*(1), 141-156.

Nicklisch, A., Shatwell, T., and Köhler, J. (2008). Analysis and modelling of the interactive effects of temperature and light on phytoplankton growth and relevance for the spring bloom. *J. Plankton Res.* 30, 75–91. doi:10.1093/plankt/fbm099.

Nixdorf, B. and Behrendt, H. (1991). Discrepancies between O_2-and ^{14}C-method resulting from measurements of primary production under consideration of vertical mixing in a shallow eutrophic lake. *Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen*, *24*(2), 1268-1271.

Phillips, J. N., and J. Myers. 1954.Growth rate of *Chlorella* in flashing light. Plant Physiol. 29: 152–161. doi:10.1104/ pp.29.2.152

Pirt, S. J. (1975). *Principles of microbe and cell cultivation*. Blackwell Scientific Publications.

Platt, T. G. C. L. et al. (1980). "Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton.".

Prasil, O., Kolber, Z., Berry, J. A., and Falkowski, P. G. (1996). Cyclic electron flow around photosystem II in vivo. *Photosynth. Res.* 48, 395–410.

Pinheiro, J. et al. (2018). *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-137.

Qin, B., Zhu, G., Gao, G., Zhang, Y., Li, W., Paerl, H. W., et al. (2010). A drinking water crisis in Lake Taihu, China: Linkage to climatic variability and lake management. *Environ. Manage.* 45, 105–112. doi:10.1007/s00267-009-9393-6.

Raven, J. A., & Beardall, J. (2003). Carbohydrate metabolism and respiration in algae. In *Photosynthesis in algae* (pp. 205-224). Springer, Dordrecht.

Raven, J. A. (2011). The cost of photoinhibition. *Physiol. Plant.* 142, 87–104. doi:10.1111/j.1399-3054.2011.01465.x.

R Core Team. (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reynolds, C. S. 2006. The Ecology of Phytoplankton. Cambridge University Press.

Richardson, K., Beardall, J., & Raven, J. A. (1983). Adaptation of unicellular algae to irradiance: an analysis of strategies. *New Phytol*, *93*(2), 157-191.

Riddle, A. M., and R. E. Lewis. 2000. Dispersion experiments in UK coastal waters. Estuar. Coast. Shelf Sci. 51: 243– 254. doi:10.1006/ecss.2000.0661

Riley, G. A. 1957. Phytoplankton of the North Central Sargasso Sea, 1950-52. Limnol. Oceanogr. 2: 252–270. doi: 10.1002/lno.1957.2.3.0252

Ross, O. N., R. J. Geider, E. Berdalet, M. L. Artigas, and J. Piera. 2011. Modelling the effect of

vertical mixing on bottle incubations for determining in situ phytoplankton dynamics. I. Growth rates. Mar. Ecol. Prog. Ser. 435: 13–31. doi:10.3354/meps09193

Ryther, J. H. (1956). Photosynthesis in the Ocean as a Function of Light Intensity. *Limnol. Oceanogr.* 1, 61–70. doi:10.4319/lo.1956.1.1.0061.

Schubert, H., and Forster, R. M. (1997). Sources of variability in the factors used for modelling primary productivity in eutrophic waters. *Hydrobiologia* 349, 75–85. doi:10.1023/A:1003097512651.

Schwaderer, A. S. et al. 2011. Eco-evolutionary differences in light utilization traits and distributions of freshwater phytoplankton. - Limnol. Oceanogr. 56: 589–598.

Schweiger, A. H., Irl, S. D. H., Steinbauer, M. J., Dengler, J., and Beierkuhnlein, C. (2016). Optimizing sampling approaches along ecological gradients. *Methods Ecol. Evol.* 7, 463–471. doi:10.1111/2041-210X.12495.

Shatwell, T., Nicklisch, A., and Köhler, J. (2012). Temperature and photoperiod effects on phytoplankton growing under simulated mixed layer light fluctuations. *Limnol. Oceanogr.* 57, 541–553. doi:10.4319/lo.2012.57.2.0541.

Siegel, D. A., S. C. Doney, and J. A. Yoder. 2002. The North Atlantic spring phytoplankton bloom and Sverdrup's criti- cal depth hypothesis. Science 296: 730–733. doi:10.1126/ science.1069174

Silvertown, J. 2004. Plant coexistence and the niche. - Trends Ecol. Evol. 19: 605–611.

Sommer, U. 1984. The paradox of the plankton: Fluctuations of phosphorus availability maintain diversity of phytoplankton in flow-through cultures. - Limnol. Oceanogr. 29: 633–636.

Sommer, U. 1985. Comparison between steady state and non-steady state competition: Experiments with natural phytoplankton. - Limnol. Oceanogr. 30: 335–346.

Sournia, A., Chrdtiennot-Dinet, M. J., & Ricard, M. (1991). Marine phytoplankton: how many species in the world ocean?.*Journal of Plankton Research*, *13*(5), 1093-1099.

Stramski, D., Rosenberg, G., & Legendre, L. (1993). Photosynthetic and optical properties of the marine chlorophyte Dunaliella tertiolecta grown under fluctuating light caused by surface-wave focusing. *Marine Biology*, *115*(3), 363-372.

Su, W. W., T. Jakob, and C. Wilhelm. 2012. The impact of nonphotochemical quenching of fluorescence on the photon balance in diatoms under dynamic light conditions. J. Phycol. 48: 336–346. doi:10.1111/j.1529- 8817.2012.01128.x

Talmy, D., Blackford, J., Hardman-Mountford, N. J., Dumbrell, A. J., and Geider, R. J. (2013). An optimality model of photoadaptation in contrasting aquatic light regimes. *Limnol. Oceanogr.* 58, 1802–1818. doi:10.4319/lo.2013.58.5.1802.

Thornley, J. H. M. (1974). Light Fluctuations and Photosynthesis. *Ann. Bot.* 38, 363–373. doi:10.1093/oxfordjournals.aob.a084820.

Thorpe, S.A. (2004). Langmuir circulation. - Annu. Rev. Fluid Mech. 36: 55-79.

Throndsen, J. 1978. Preservation and storage. Phytoplankton manual.

Toepel, J., Gilbert, M., and Wilhelm, C. (2004). Can Chlorophyll-a in-vivo fluorescence be used for quantification of carbon-based primary production in absolute terms? *Arch. fur Hydrobiol.* 160, 515–526. doi:10.1127/0003-9136/2004/0160-0515.

Utermöhl, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. - Mit. Int. Ver. Theo. Angew. Limnol. 9: 1-38.

van de Poll, W. H., R. J. W. Visser, and A. G. J. Buma. 2007. Acclimation to a dynamic irradiance regime changes excessive irradiance sensitivity of *Emiliania huxleyi* and *Thalassiosira weissflogii*. Limnol. Oceanogr. 52: 1430–1438. doi:10.4319/lo.2007.52.4.1430

Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., & Garnier, E. (2007). Let the concept of trait be functional!. *Oikos*, *116*(5), 882-892.

Wagner, H., Jakob, T., and Wilhelm, C. (2006). Balancing the energy flow from captured light to biomass under fluctuating light conditions. *New Phytol.* 169, 95–108. doi:10.1111/j.1469-8137.2005.01550.x.

Walsh, P., & Legendre, L. (1983). Photosynthesis of natural phytoplankton under high frequency light fluctuations simulating those induced by sea surface waves 1. *Limnology and Oceanography*, *28*(4), 688-697.

Wang, L., Q. Cai, Y. Xu, L. Kong, L. Tan, and M. Zhang. 2011a. Weekly dynamics of phytoplankton functional groups under high water level fluctuations in a subtropical reservoir-bay. Aquat. Ecol. 45: 197–212. doi:10.1007/ s10452-010-9346-4

Wang, L., Q. Cai, M. Zhang, L. Tan, and L. Kong. 2011b. Longitudinal patterns of phytoplankton distribution in a tributary bay under reservoir operation. Quat. Int. 244: 280–288. doi:10.1016/j.quaint.2010.09.012

Webb, W. L., Newton, M., and Starr, D. (1974). Carbon dioxide exchange of *Alnus rubra* - A mathematical model. *Oecologia* 17, 281–291. doi:10.1007/BF00345747.

Weger, H. G., Herzig, R., Falkowski, P. G., and Turpin, D. H. (1989). Respiratory losses in the light in a marine diatom: Measurements by short-term mass spectrometry. *Limnol. Oceanogr.* 34, 1153–1161.

Yoon, H. S., Hackett, J. D., Ciniglia, C., Pinto, G., and Bhattacharya, D. (2004). A Molecular Timeline for the Origin of Photosynthetic Eukaryotes. *Mol. Biol. Evol.* 21, 809–818. doi:10.1093/molbev/msh075.

Zlotnik, I., and Dubinsky, Z. (1989). The effect of light and temperature on DOC excretion by phytoplankton. *Limnol. Oceanogr.* 34, 831–839. doi:10.4319/lo.1989.34.5.0831.

Acknowledgements

There was a time when I was making experiments with no control treatments, no p-values, no fancy machines nor dangerous chemicals with my Dad. These experiments are by far the ones that I cherish the most. Since then, things have changed a lot. But my parents always remained here for me. Thus, it is only natural to first thank my Mom and Dad for their constant support and love. Much less considerate, I thank my Brother and Sister who have always kept reminding me that I am the youngest kid of the family. I hope these 150 pages about phytoplankton will make them understand that I am not a fish specialist and that my title is not *"Dr. Poisson"* meaning *"Dr. Fish".* Though, it is still better that my grandma thinking I will be a medical doctor for algae… Mamie, this PhD is for you!

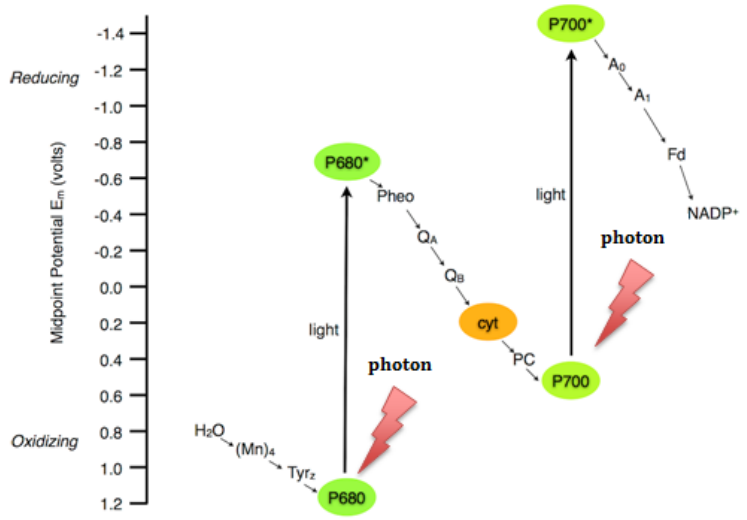
This work could not have been undertaken without my supervisor Dr. Jan Köhler. I thank Jan for fruitful and intellectually challenging discussions about ecology but also life in general. Thank you for your trust and for giving me independence in my work. I will never forget the beautiful places we have visited together in China – particularly our stay in Hongcun village, place I thought we would never be able to leave.
I also want to thank my supervisor Prof. Dr. Ursula Gaedke who was always there for me and whose work triggered my passion of phytoplankton many years ago during my bachelor studies. I am grateful for your support during my doctorate, tips and tricks for publication and for your constant tracking of my progresses.

I acknowledge many other scientists who helped me at one point or another during my PhD: Guntram Weithoff during my teaching weeks at Potsdam University, Thomas Mehner for scientific writing advises, Kirsten Pohlmann for the doctoral program at IGB, Tom Shatwell for his crash course on nonlinear models and all scientists with whom I collaborated. I do not forget the valuable contributions of Katrin Preuß, Barbara Stein, Paul Genz, Berta Ortiz-Crespo, Julia Kionka, Kate Laskowski, Ramsy Agha, Grit Siegert, Amir Bazyar, Katrin Lehmann and all members of the Köhler-Hilt group.

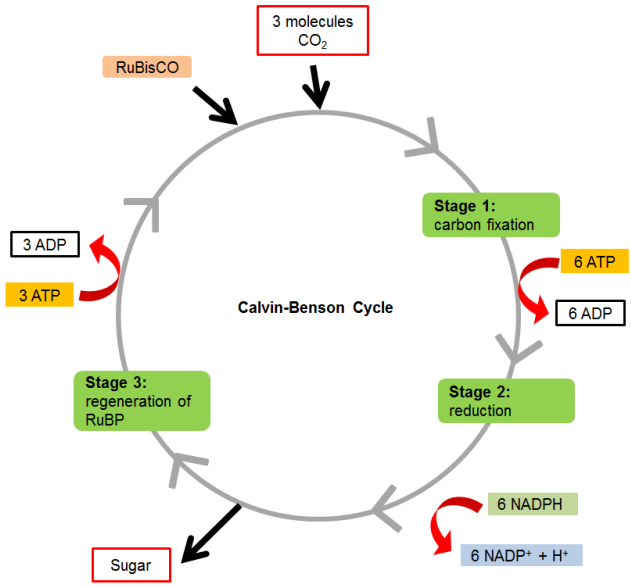
Finally, many thanks to all members of the Kinderzimmer, Tarun, Jason, Ramsy, Marta, Paul, Mikael, Mauro, Karin, Garabet, Tim, Mandy, Guillaume and of course Merve for endless support.

Supplementary material

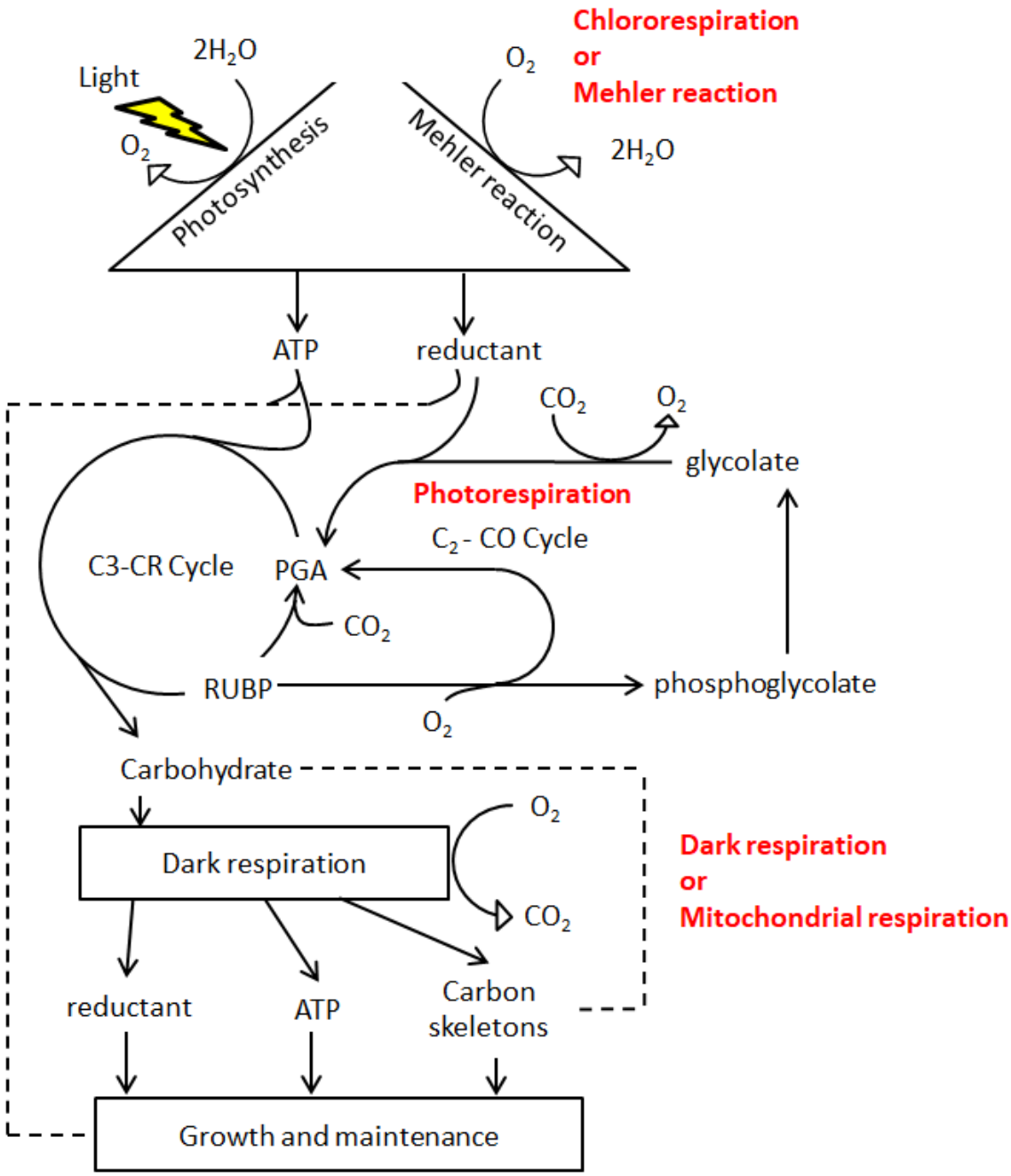
1. General introduction



Appendix - Figure 1. The "Z-scheme" for photosynthetic electron transport. It explains how the photosynthetic electron transport chain works. Electrons move towards the oxydo-reduction potential. P700 (PSI) and P680 (PSII) are named after their wavelength peak absorption of light. It is interesting to note that the pigment composition of antennae may differ between species and also between both photosystems (Falkowski and Raven 2007).

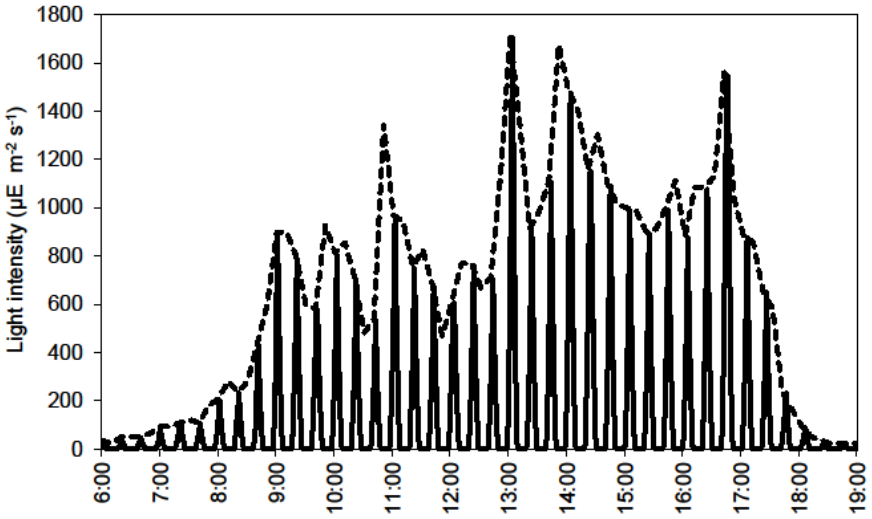


Appendix- Figure 2. The light-independent Calvin-Benson cycle (Falkowski and Raven 2007).



Appendix- Figure 3. Schematic representation of respiratory processes: dark respiration, photorespiration and Mehler reaction.

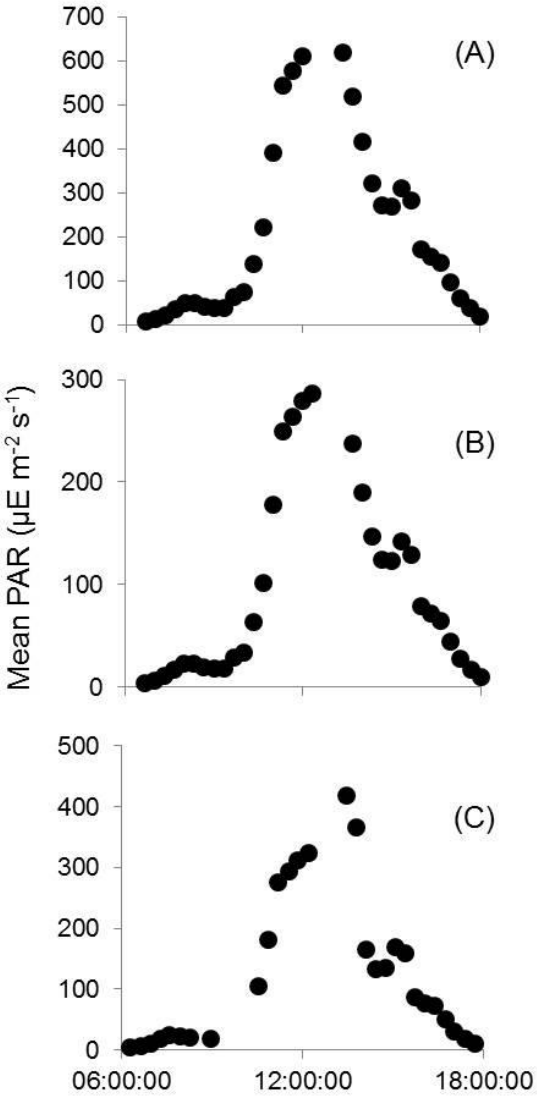
2. Chapter 2



Appendix - Figure 1. Example of diurnal course of light intensity at the water surface (dotted line) and experienced by phytoplankton under complete water column mixing (0–180 cm) (full line) for the two extreme light supply treatments taken at the Lake station, September 7th 2016 (attenuation coefficient = 4.97 m^{-1}). Phytoplankton received 3 E m^{-2} d^{-1} (100 % PAR relative) at the surface versus 0.93 E m^{-2} d^{-1} (30.9 % PAR relative) for the case of full over-turn.

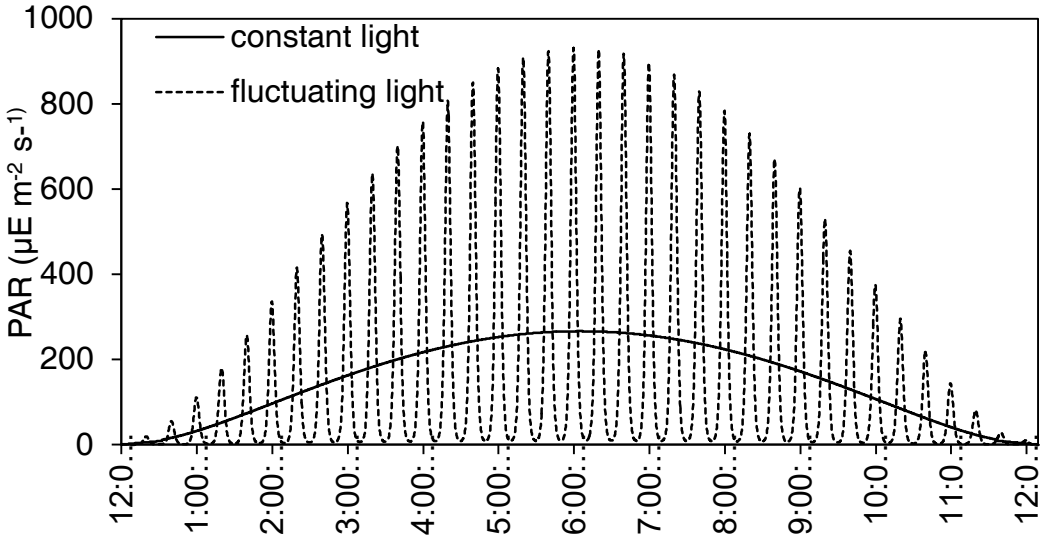
Appendix - Table 1. Daily photosynthetically active radiation PAR (E m^{-2} d^{-1}) received by each treatment over the whole experimental period. Daily PAR exposure was corrected for shade, light attenuation of the lake, transmittance of the incubation bottles and vertical motion of moved algae.

		0 - 0.5m		0 - 1m		0 - 1.8m	
	surface	Fixed	Moved	Fixed	Moved	Fixed	Moved
7 Sept	3.00	1.42	1.65	0.53	1.12	0.03	0.93
8 Sept	7.19	4.02	4.19	1.86	2.91	0.18	2.25
9 Sept	10.23	4.67	5.58	1.64	3.78	0.07	2.91
10 Sept	1.65	0.88	0.94	0.38	0.65	0.03	0.50
11 Sept	1.68	0.74	0.91	0.25	0.61	0.01	0.47
13 Sept	12.58	6.78	7.22	2.97	4.99	0.57	3.90
14 Sept	1.36	0.48	0.57	0.17	0.39	0.02	0.31
15 Sept	2.78	1.39	1.55	0.55	1.06	0.09	0.81
16 Sept	2.58	1.68	1.82	0.71	1.25	0.13	0.99
Average	4.78	2.45	2.71	1.01	1.86	0.13	1.45

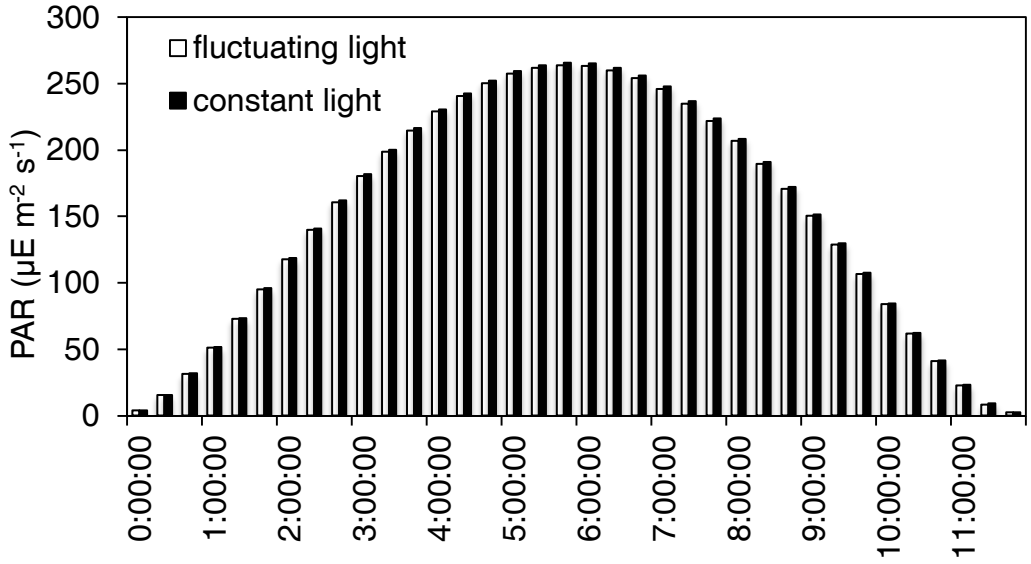


Appendix - Figure 2. Mean PAR intensities received at the 20 min timescale on September 9th for samples incubated at (A) the surface, (B) 20 cm depth and (C) vertically mixed from 0 to 50 cm depth. Light intensities increased in the morning from sunrise until 1 pm and decreased in the afternoon from 1 pm until sunset.

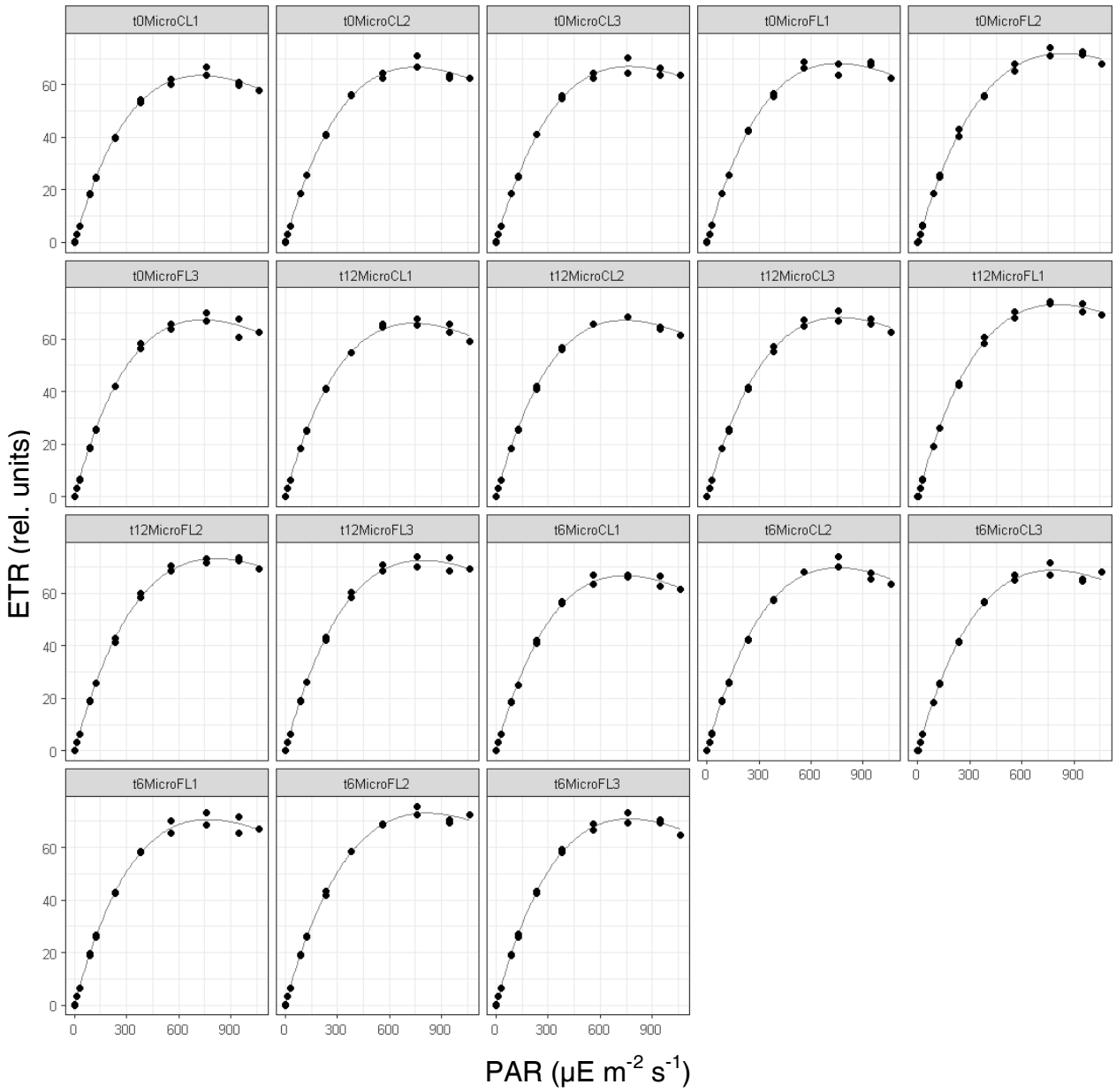
3. Chapter 3



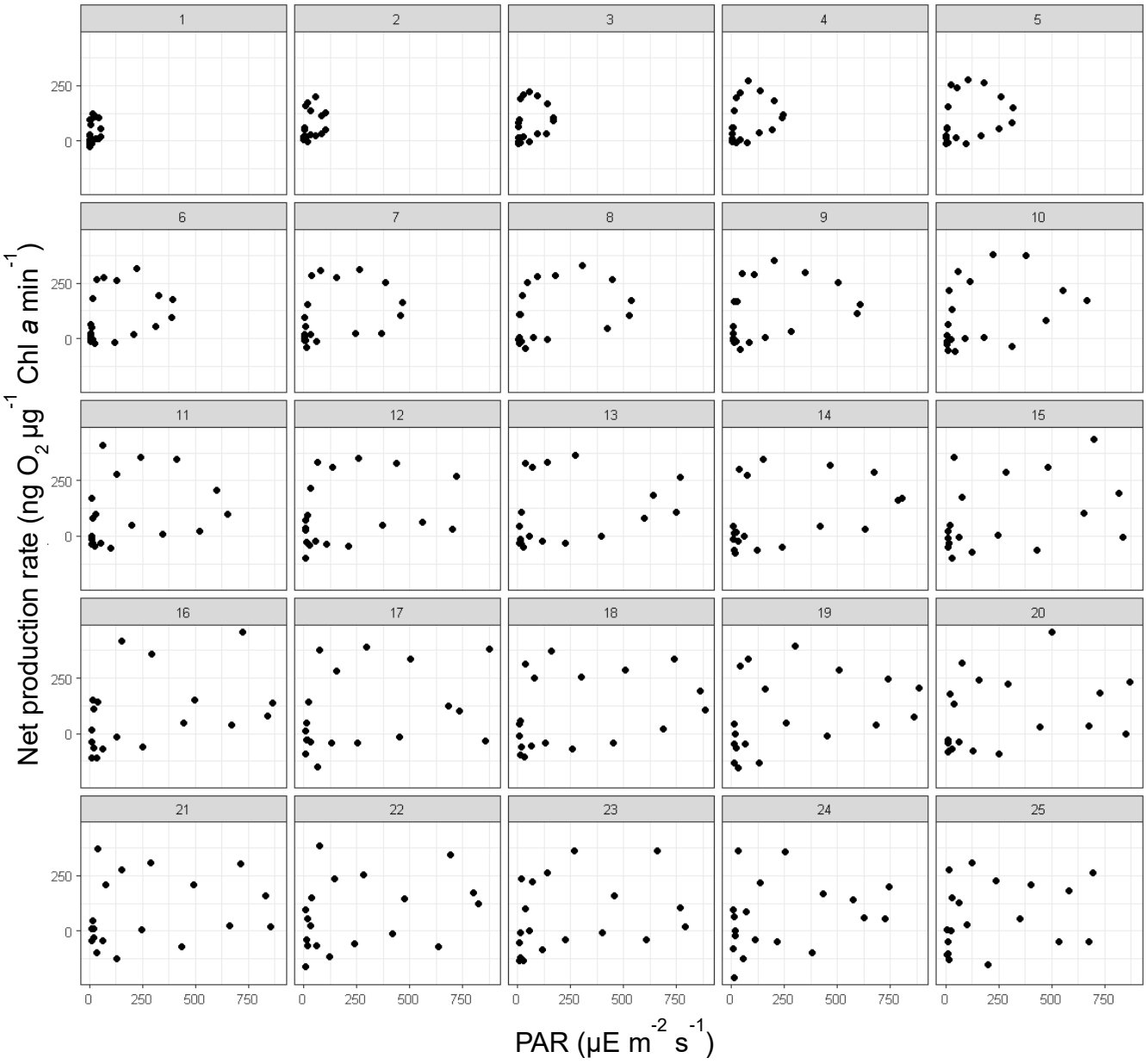
Appendix - Figure 1. Diurnal course (12:12 photoperiod) of the photosynthetically active radiation (PAR) experienced by *Microcystis* under constant (full line) and fluctuating light (dashed line).



Appendix - Figure 2. Twenty minutes mean photosynthetically active radiation (PAR) experienced by *Microcystis* under constant (full bars) and fluctuating light (empty bars).



Appendix - Figure 3. ETR-light relationships measured in each replicates (1, 2, 3) at the beginning (t0), after 6 hours (t6) and 12 hours (t12) of constant (CL) and fluctuating light incubation (FL). Electron transport rates measured at increasing (1 to 1062 µE m^{-2} s^{-1}) and decreasing (946 to 14 µE m^{-2} s^{-1}) irradiance levels are here fitted together with the Platt model.



Appendix - Figure 4. Duplicates averaged net oxygen production rates calculated every minute under fluctuating light for every consecutive 20 min cycles.

4. Chapter 4

Table A1. Species composition of the isolated Lake TaiHu phytoplankton community during the experiment. *Chloro*: Chlorophyceae; *Bacill*: Bacillariophyceae; *Cyano*: Cyanophyceae; *Zygn*: Zygnematophyceae.

Class	Species
Chloro	*Actinastrum hantzschii*
-	*Coelastrum astroideum*
-	*Coelastrum microporum*
-	*Crucigenia fenestrata*
-	*Crucigenia quadrata*
-	*Crucigeniella apiculata*
-	*Didymocystis spec.*
-	*Elakatothrix spec.*
-	*Eudorina spec.*
-	*Lagerheimia ciliata*
-	*Lagerheimia wratislavensis*
-	*Micractinium pusillum*
-	*Monoraphidium arcuatum*
-	*Monoraphidium contortum*
-	*Monoraphidium griffithii*
-	*Oosystis spp.*
-	*Pediastrum boryanum*
-	*Pediastrum duplex*
-	*Pediastrum simplex*
-	*Pediastrum tetras*
-	*Planktonema (Binuk.) lauterbornii*
-	*Planktosphaeria gelatinosa*
-	*Raphidocelis spec.*
-	*Scenedesmus acuminatus*
-	*Scenedesmus bijuga*
-	*Scenedesmus communis*
-	*Scenedesmus falcatus*
-	*Scenedesmus intermedius*
-	*Scenedesmus maximus*
-	*Scenedesmus sempervirens*
-	*Scenedesmus serratus*
-	*Scenedesmus subspicatus*
-	*Scenedesnus spp*
-	*Schroederia indica*
-	*Schroederia setigera*
-	*Schroederia spec.*
-	*Tetraedron caudatum*
-	*Tetraedron minimum*
Bacill	*Aulacoseira granulata*
-	*Aulacoseira spp.*
-	*Cyclotella pseudostelligera*
-	*Nitzschia acicularis*
-	*Nitzschia fonticola*
-	*Nitzschia spp.*
Cyano	*Anabaena flos- aquae*
-	*Anabaena spec., gerade*
-	*Aphanizomenon issatschenkoi*
-	*Chroococcus turgidus*
-	*Geitlerinema unsure*
-	*Limnothrix spec.*
-	*Merismopedia spec*
-	*Microcystis spec.*
-	*Oscillatoria spp.*
-	*Planktothrix spp*
-	*Raphidiopsis curvata*
-	*Raphidiopsis spec.*
Zygn	*Closterium acutum v. variabile*

Table A2. Daily photosynthetically active radiation (E m-2 d-1) received by each treatment over the whole experiment period. Daily PAR exposure was corrected for shade, light attenuation of the lake, transmittance of the incubation bottles and vertical motion of moved algae.

		0 - 0.5m		0 - 1m		0 - 1.8m	
	surface	Fixed	Moved	Fixed	Moved	Fixed	Moved
7 Sept	3.00	1.42	1.65	0.53	1.12	0.03	0.93
8 Sept	7.19	4.02	4.19	1.86	2.91	0.18	2.25
9 Sept	10.23	4.67	5.58	1.64	3.78	0.07	2.91
10 Sept	1.65	0.88	0.94	0.38	0.65	0.03	0.50
11 Sept	1.68	0.74	0.91	0.25	0.61	0.01	0.47
13 Sept	12.58	6.78	7.22	2.97	4.99	0.57	3.90
14 Sept	1.36	0.48	0.57	0.17	0.39	0.02	0.31
15 Sept	2.78	1.39	1.55	0.55	1.06	0.09	0.81
16 Sept	2.58	1.68	1.82	0.71	1.25	0.13	0.99
Average	4.78	2.45	2.71	1.01	1.86	0.13	1.45

Table A3. Averaged relative contributions of the main phytoplankton groups to the total biovolumes under constant and fluctuating light across the entire experimental period.

	Constant light			Fluctuating light		
	Cyanobacteria	Diatoms	Chlorophyceae	Cyanobacteria	Diatoms	Chlorophyceae
Minimal contribution	0.10	0.38	0	0.14	0.55	0
Maximal contribution	0.46	0.90	0.21	0.43	0.85	0.04
Mean ± Standard deviation	0.27 ± 0.11	0.69 ± 0.14	0.04 ± 0.05	0.25 ± 0.09	0.73 ± 0.1	0.02 ± 0.01

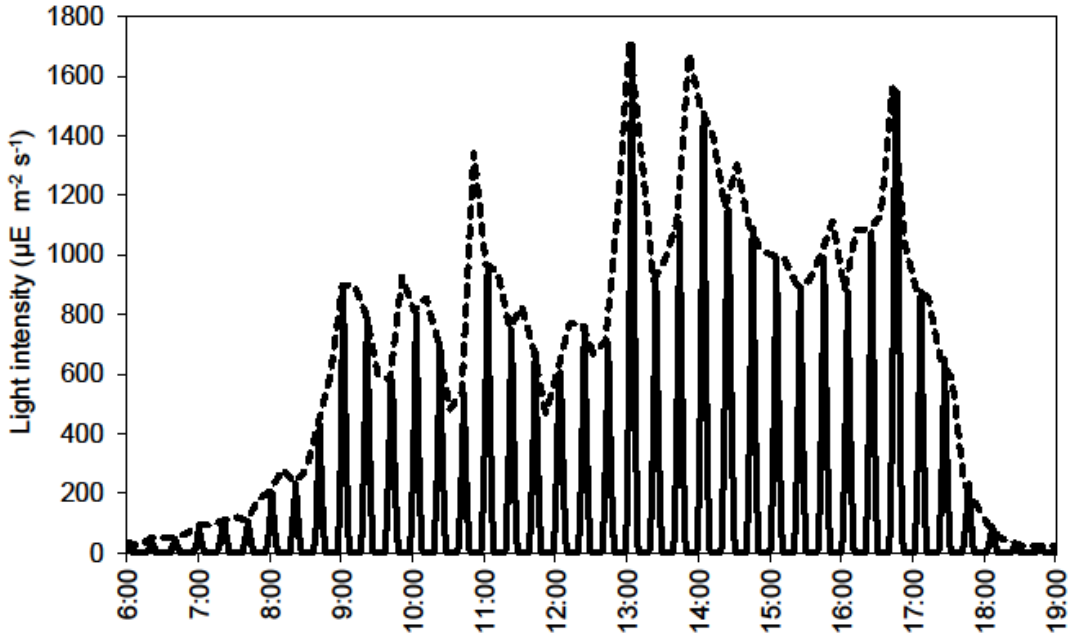


Figure A1. Example of diurnal course of light intensity at the water surface (dotted line) and experienced by phytoplankton under complete water column mixing (0–1.8 m) (full line) for the two extreme light supply treatments taken at the Lake station, 7 September 2016 (attenuation coefficient = 4.97 m-1). Phytoplankton received 3 E m-2 day-1 (100% PAR relative) at the surface versus 0.93 E m-2 day-1 (30.9% PAR relative) for the case of full over-turn.

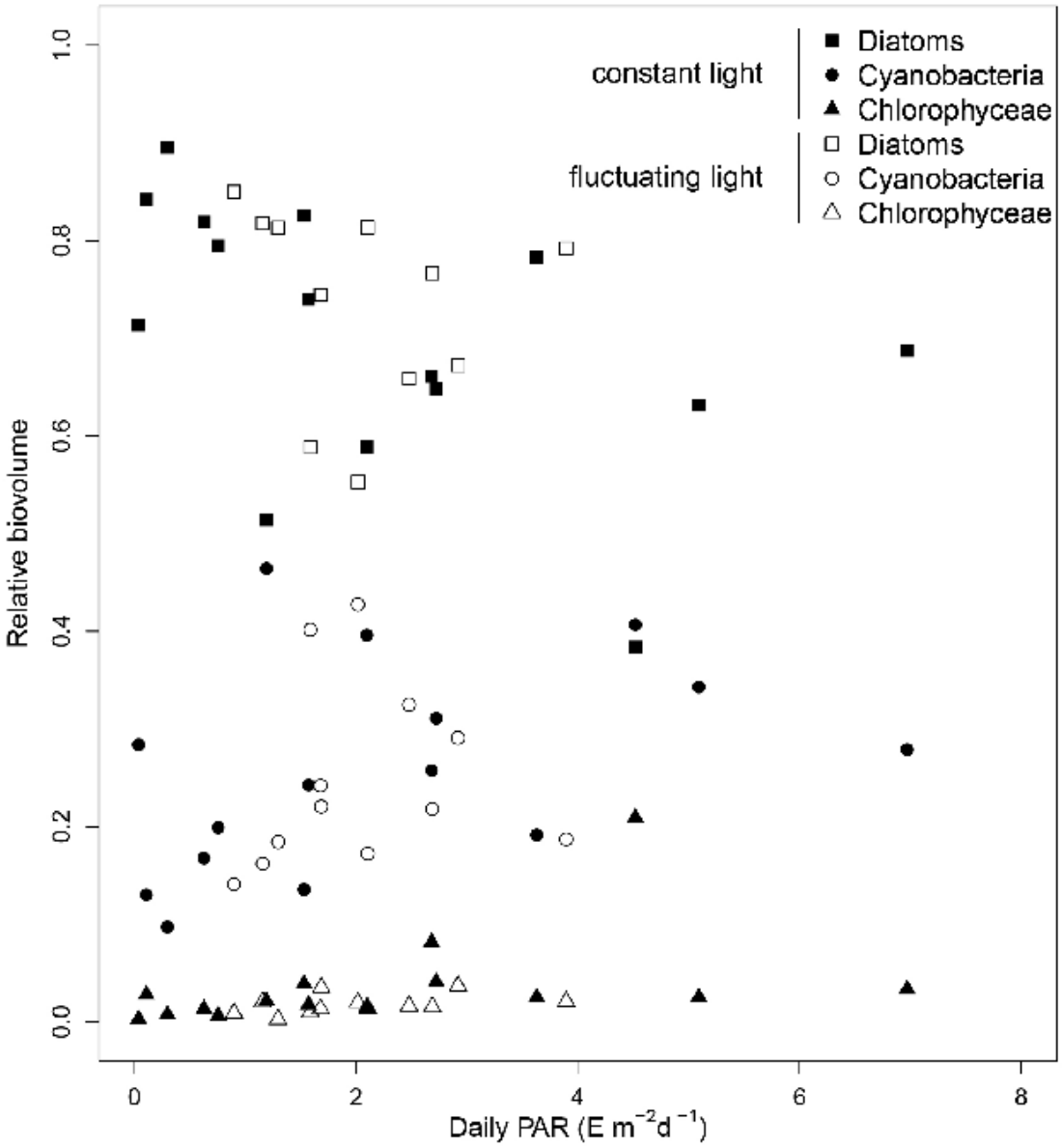


Figure A2. Light-dependency of the relative biovolumes of diatoms, cyanobacteria and chlorophyceae to the total biovolume, under fluctuating (open symbols) and constant light (closed symbols). Averages over [day 0 – day 1] and [day 2 – end experiment] represented the relative contributions at day 2 and at the end of the experiment respectively.

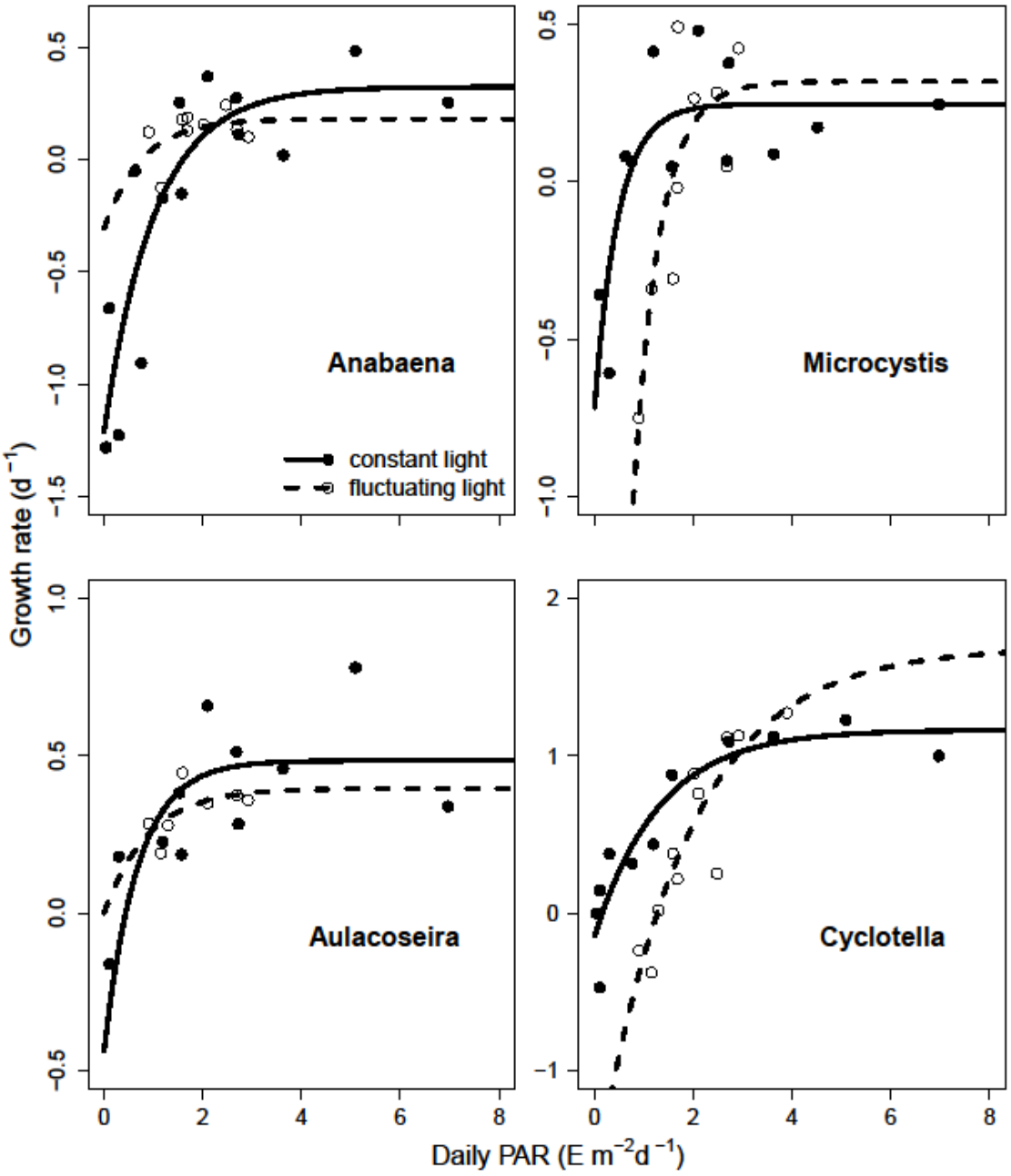


Figure A3. Species-specific growth-light relationships of *Anabaena flos-aquae*, *Microcystis* spp., *Aulacoseira granulata* and *Cyclotella pseudostelligera* under fluctuating and constant light.

List of publications

- Alexandrine Pannard, **Alexis Guislain**, Marion Chorin, Stéphane Mahé, Guillaume Bouger, Alain Crave, Bertrand Le Rouzic & Myriam Bormans (2018). Phosphorus more than temperature controls the phytoplankton community in a deep quarry lake: a combined field and laboratory approach. *Inland Waters*, *8*(1), 22-35.

- Jan Köhler, Lan Wang, **Alexis Guislain** & Tom Shatwell (2018). Influence of vertical mixing on light-dependency of phytoplankton growth. *Limnology and Oceanography*, *63*(3), 1156-1167.

- **Alexis Guislain**, Beatrix Elisabeth Beisner & Jan Köhler (2019). Variation in species light acquisition traits under fluctuating light regimes: implications for non-equilibrium coexistence. *Oikos*, *128*(5), 716-728.

- **Alexis Guislain** & Jan Köhler (under review, Frontiers in Freshwater Science). Interplay between photosynthesis, respiration and growth of phytoplankton communities under vertical mixing.

- **Alexis Guislain** & Jan Köhler (in prep.) How does the cyanobacterium *Microcystis aeruginosa* respond to fluctuating light? A minute-based analysis of photosynthesis and respiration.

- Anne Lyche Solheim, Hege Gundersen, Jannicke Moe, Ute Mischke, Birger Skjelbred, Jessica Richardson, Laurence Carvalho, Heleen de Wit, Stella Berger, Jens Nejstgaard, Darren Giling, **Alexis Guislain**, Andreas Jechow…(in prep.) Cyanobacteria response to browning and nutrient enrichment in stratified lakes (EU-funded MARS project).

- **Alexis Guislain**, Jutta Fastner & Jan Köhler (in prep.) Effects of fluctuating light on toxin production of *Microcystis aeruginosa.*

** Underlined papers are included in the thesis.*

List of presentations

2019

- 11th Symposium for European Freshwater Sciences, Zagreb, Croatia. Talk. (Speaker: López Moreira M. G. A.) *Top-down control modulates interactions between primary producers in lakes undergoing eutrophication and browning: a mesocosm modelling study.*
- ALSO meeting, San Juan, Puerto-Rico. Talk. (Speaker: Köhler J.) *Influence of vertical mixing on light dependency of photosynthesis, respiration and growth of phytoplankton communities.*

2018

- Final IGB presentation, Berlin, Germany. Talk. *How does vertical mixing impact the eco-physiology of phytoplankton?*
- Final SIGN project meeting, Chinese Research Academy of Environmental Sciences (CRAES), Beijing, China. Talk. *How does vertical mixing impact the eco-physiology of phytoplankton? - Implications for Lake TaiHu management.*
- IGB group meeting, Berlin, Germany. Talk. *A way to counter pseudo-replication in regression analysis: The nonlinear mixed effects models nlme() command in R.*

2017

- Group seminar, Potsdam University, Germany. Talk. *Effects of fluctuating light on phytoplankton growth – Application in the light-limited lake TaiHu (China).*
- 11th International Phycological Congress (IPC), Szczecin, Poland. Poster. *How does fluctuating light impact growth, photosynthesis and respiration of phytoplankton communities?*
- 9th International Shallow lakes conference, Merida, Mexico. Talk. *Effects of vertical mixing on phytoplankton development in the shallow lake TaiHu (China).*
- IGB department meeting, Berlin, Germany. Talk. *Effects of fluctuating light on the eco-physiology of phytoplankton - Application in the light-limited lake TaiHu (China).*
- SIGN meeting, Karlsruhe, Germany. Talk. *Monitoring station of lake TaiHu NIGLAS.*
- SIGN meeting, NIGLAS, Nanjing, China. Talk. *Effects of vertical mixing on phytoplankton development in lake TaiHu.*

2016

- PhD project presentation, IGB, Berlin, Germany. Talk. *Effects of fluctuating light on photosynthesis and growth of phytoplankton.*
- Research group meeting, IGB, Berlin, Germany. Talk. *Effects of wind-induced mixing on phytoplankton development in TaiHu - first field experiment.*
- Research group meeting, IGB, Berlin, Germany. Talk. *Influence of vertical mixing on phytoplankton development.*

Statement of academic integrity

I hereby declare, that the dissertation entitled "Eco-physiological consequences of fluctuating light on phytoplankton" is my own work. No sources other than those indicated have been used. All collaboration that has taken place with other researchers is indicated. This thesis has not been submitted for a doctoral degree at any other institution.

Hiermit erkläre ich, dass die Dissertation mit dem Titel "Eco-physiological consequences of fluctuating light on phytoplankton" meine eigene Arbeit ist. Sie wurde nur unter der Verwendung der angegebenen Hilfen und Hilfsmittel angefertigt. Kooperationen mit anderen Wissenschaftlern wurden angegeben. Diese Dissertation wurde an keiner anderen Universität eingereicht.

Berlin, November 11th 2019

Curriculum vitae

Alexis GUISLAIN
alexis.guislain@gmail.com

Research experience

Since 2015 PhD student – Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) & University of Potsdam, Germany
"Effects of fluctuating light on photosynthesis and growth of phytoplankton" (Advisors: J. Köhler, U. Gaedke) - Sino-German Water supply network (SIGN) project funded by BMBF

2014 MSc (2nd year) thesis - UMR ECOBIO 6553 Rennes 1
"Response of lacustrine phytoplankton species to fluctuating phosphorus supply" (Advisors: A. Pannard, A.J. Francez)

2013 MSc (1st year) thesis - UMR ECOBIO 6553 Rennes 1
"Decoupling the control factors of a quarry lake phytoplankton community" (Advisors: A. Pannard, M. Bormans)

2012 Bachelor thesis - UMR ECOBIO 6553 Rennes 1
"Acclimatization strategies to the toxic stresses of an invasive freshwater gastropoda: *Physa acuta*" (Advisor: E. Lance)

Education

Since 2015 PhD student - Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) & University of Potsdam, Germany

2012-2014 MSc in Evolutionary, Functional and Behavioural Ecology - University of Rennes 1, France

2009-2012 Bachelor in Biology of organisms - University of Rennes 1, France

Teaching & Supervision

Teaching: Practical course in plankton ecology (for MSc and BSc), University of Potsdam.
Supervision: 2 MSc and 1 BSc students.

Research projects

Since 2015 Sino-German Water supply network project (SIGN), funded by BMBF. Cooperation between German and Chinese partners from the industry, research and development to improve water quality in the TaiHu region, China.

2015 MARS Project: Managing Aquatic ecosystems and water resources under multiple stress, funded by the European Union under the 7th Framework Programme.

Publications in peer-reviewed journals

Guislain, A., Beisner, B. E., & Köhler, J. (2019). Variation in species light acquisition traits under fluctuating light regimes: implications for non-equilibrium coexistence. *Oikos*, *128*(5), 716-728.

Köhler, J., Wang, L., **Guislain, A**., & Shatwell, T. (2018). Influence of vertical mixing on light-dependency of phytoplankton growth. *Limnology and Oceanography*, *63*(3), 1156-1167.

Pannard, A., **Guislain, A**., Chorin, M., Mahé, S., Bouger, G., Crave, A., ... & Bormans, M. (2018). Phosphorus more than temperature controls the phytoplankton community in a deep quarry lake: a combined field and laboratory approach. *Inland Waters*, *8*(1), 22-35.

Presentations

2018

- Final IGB presentation, Berlin, Germany. Talk. *How does vertical mixing impact the eco-physiology of phytoplankton?*
- Final SIGN project meeting, Chinese Research Academy of Environmental Sciences (CRAES), Beijing, China. Talk. *How does vertical mixing impact the eco-physiology of phytoplankton? - Implications for Lake TaiHu management.*
- IGB group meeting, Berlin, Germany. Talk. *A way to counter pseudo-replication in regression analysis: The nonlinear mixed effects models nlme() command in R.*

2017

- Group seminar, Potsdam University, Germany. Talk. *Effects of fluctuating light on phytoplankton growth – Application in the light-limited lake TaiHu (China).*
- 11th International Phycological Congress (IPC), Szczecin, Poland. Poster. *How does fluctuating light impact growth, photosynthesis and respiration of phytoplankton communities?*

- 9th International Shallow lakes conference, Merida, Mexico. Talk. *Effects of vertical mixing on phytoplankton development in the shallow lake TaiHu (China).*
- IGB department meeting, Berlin, Germany. Talk. *Effects of fluctuating light on the eco-physiology of phytoplankton - Application in the light-limited lake TaiHu (China).*
- SIGN meeting, Karlsruhe, Germany. Talk. *Monitoring station of lake TaiHu NIGLAS.*
- SIGN meeting, NIGLAS, Nanjing, China. Talk. *Effects of vertical mixing on phytoplankton development in lake TaiHu.*

2016

- PhD project presentation, IGB, Berlin, Germany. Talk. *Effects of fluctuating light on photosynthesis and growth of phytoplankton.*
- Research group meeting, IGB, Berlin, Germany. Talk. *Effects of wind-induced mixing on phytoplankton development in TaiHu - first field experiment.*
- Research group meeting, IGB, Berlin, Germany. Talk. *Influence of vertical mixing on phytoplankton development.*

Other

Languages: French, English, Spanish, German

Driving licence

First aid certificate