Growth vs. light-capture in PhycoCyanin and PhycoErythrin-rich picocyanobacteria, across photic regimes and growth phases Alternate: Long & low vs. high & short & fast; growth yields, light and photoperiods in PhycoCyanin and PhycoErythrin-rich picocyanobacteria

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**Running head:** *Picocyanobacteria across photic regimes*

# Abstract

The genus *Synechococcus* occurs between tropical and arctic zones with long-term scenarios forecasting range expansions of this picocyanobacteria into new photic regimes. We found that PC-rich and PE-rich strains grew most favorably under low light and 24-hour photoperiod, despite the cumulative diel PUR dose being equivalent to conditions where the light intensity was higher and the photoperiod was shorter. Under optimal conditions, PE-rich *Synechococcus* sp. achieved the highest recorded cyanobacterial chlorophyll-specific exponential growth rate (µ) of 4.5 d−1. PE-rich strains demonstrated high ability to modulate light capacity whereas PC-rich strains maintained a consistent PUR/PAR ratio across increasing cumulative diel PAR dose. We found, for the first time, that PC-rich and PE-rich picocyanobacteria show consistent patterns of effective absorption cross section for PSII photochemistry (σPSII′; nm2 quanta−1), versus increasing cumulative diel PAR doses. The σPSII′ excited through phycobilisome absorbance at 590 nm were positively correlated with phycobiliprotein to Chl *a* ratio however, in the exponential growth phase, high variability was observed, likely related to regulatory control of σPSII′ beyond pigment composition. Here, for the first time, we calibrated the *JV*PSII estimator to absolute rates of electron transport using parallel measures of oxygen evolution (µmolO2 L−1 s−1), captured simultaneously to the Fast Repetition Rate fluorometry (FRRf) measures. Within each strain, µ showed consistent saturating responses to increasing cumulative diel PSII electron flux (*JV*PSII; µmol e− µmol Chl *a*−1 d−1) indicating that picocyanobacteria have the potential to expand into new photic regimes while PE-rich picocyanobacteria may emerge as the dominant phytoplankter.

# Introduction

The photic regime, comprised of Photosynthetically Active Radiation (PAR), spectral quality, and photoperiod, is a pivotal influence on the growth and productivity of phytoplankton within aquatic ecosystems. PAR refers to the spectral range of solar radiation, approximately 400-700 nm, that is capable of driving photosynthesis. The availability and distribution of PAR in aquatic ecosystems is influenced by cloud cover, water depth, and light attenuation due to water turbidity and suspended particles, including phytoplankton cells (Field et al. 1998; Torremorell et al. 2009). Photosynthetically Usable Radiation (PUR), in turn is the fraction of PAR that can be absorbed for photosynthesis by pigments of a given cyanobacteria or algae (Morel 1978). PUR thus depends upon the interaction of PAR, and the phytoplankter expression of genomic capacities for light capture (Moejes et al. 2017). Cyanobacteria also respond to changes in photoperiod, which serves as a key environmental cue for photosynthesis, growth, reproduction, and nutrient assimilation (LaRoche and Robicheau 2022). Thus, in polar regions, characterized by prolonged periods of wintertime darkness and continuous daylight during summer, cyanobacteria encounter unique challenges. Light is the primary limitation on biomass production in winter, suppressing cyanobacteria growth and metabolic activity, whereas extended daylight in summer boosts photosynthetic activity (Arrigo 2014). In temperate regions, seasonal variation in light-limitation is less pronounced, but cyanobacteria are still influenced by daily and seasonal fluctuations, with a contrast between more favorable conditions for cyanobacteria growth in spring and summer, compared to fall and winter (Huisman et al. 2002; Holtrop et al. 2021). In the tropics, daylight hours remain nearly constant throughout the year (Behrenfeld et al. 2006), and cyanobacteria productivity is rather controlled by nutrients resupplied into the euphotic zone (Li et al. 2015), and mortality through viral lysis (Ortmann et al. 2002) and zooplankton grazing (Christaki et al. 1999).

*Synechococcus*, a diverse genus of picocyanobacteria, exhibits a distribution spanning diverse geographical regions (Flombaum et al. 2013), with strains demonstrating a remarkable range of adaptations to environmental conditions (Śliwińska-Wilczewska et al. 2018a; Aguilera et al. 2023). *Synechococcus* capacities to thrive across diverse marine and freshwater habitats positions it as a pivotal agent in energy and nutrient transfer within food webs, connecting the microbial loop with higher trophic levels, offering direct sustenance to grazers, including zooplankton and small fish (Li 1995). As one of the two dominant picocyanobacterial genera in oceanic waters, *Synechococcus* contribute significantly to light attenuation and light availability for other photosynthetic marine organisms, thereby influencing ocean colour and allowing satellite detection of *Synechococcus*-rich communities (Xi et al. 2020). General relations among optical absorption spectra and pigment compositions have been used to determine diagnostic pigment indices of major phytoplankton functional types (Hirata et al. 2011). Modeling suggests that *Synechococcus* abundance and ranges will increase due to climate warming (Flombaum et al. 2013). The projected changes may vary geographically and may include shifts in the spatial distribution of the main picocyanobacteria, as well as changes in the proportions among *Synechococcus* sp. lineages (Six et al. 2021), potentially pushing lineages into new photic regimes. Synechococcus exhibits significant phenotypic diversity across lineages, encompassing strains rich in phycobiliprotein pigments, phycorrythrin (PE-rich) or phycocyanin (PC-rich) (Haverkamp et al. 2009; Aguilera et al. 2023). Phycobiliprotein pigments are pivotal for light absorption during photosynthesis and confer distinctive colours to the picocyanobacteria. The disparate light preferences between PC-rich and PE-rich *Synechococcus* sp. strains influence their ecological niches. PC-rich strains thrive in environments with elevated light levels, such as surface waters and coastal regions. PE-rich strains exhibit adaptation to lower-light conditions, primarily inhabiting the deeper layers of the water column. PC-rich and PE-rich *Synechococcus* sp. strains thus predominantly occupy complementary habitats (Six et al. 2007; Haverkamp et al. 2009; Six et al. 2021), although differential responses of *Synechococcus* lineages to photoperiod, have not been studied in detail, except for thermophilic PC-rich *Synechococcus* PCC 6715 (Klepacz-Smółka et al. 2020).

Cyanobacteria growth includes lag, exponential growth, stationary, and death phases (Reynolds 2006). During the lag phase, cyanobacteria acclimate to the environment and prepare for active growth by synthesizing essential cellular components. Exponential growth phase is marked by cell division and biomass accumulation, fueled by nutrient and light availability. If growth is limited by declining nutrients, by light, or by accumulation of inhibitory factors, algae enter stationary phase, characterized by a balance between cell division and death, leading to a plateau in population. The death phase occurs when cyanobacteria cell death outruns division, leading to net decomposition, contributing to nutrient recycling in aquatic ecosystems (Reynolds 2006). Moreover, Schuurmans et al. (2017) proposed an additional phase between the exponential and stationary phases of picocyanobacteria growth, which is often neglected in physiological studies. Herein, we examined the physiological responses of PC-rich and PE-rich *Synechococcus* sp. in this phase, which we termed the pre-stationary phase of growth.

Picocyanobacteria are the most abundant phytoplankters in aquatic ecosystems and are crucial to the optical properties of ocean water, by influencing its colour and transparency. PC-rich and PE-rich *Synechococcus* sp. may have different costs and physiological strategies for growth under different photic regimes, which could drive spatial and temporal variability of picocyanobacteria biomass and community composition, in current and potential future aquatic habitats. Therefore, our aim was to determine whether photic regimes and growth phases differentially affect growth and light-capture, between representative PC-rich and PE-rich *Synechococcus* sp.

# Materials and Methods

## Culture condition and experimental setup

Two xenic PhycoCyanin(PC)-rich (CCBA\_056 or CCBA\_077) strains and two PhycoErythrin(PE)-rich (CCBA\_048 or CCBA\_127) strains of *Synechococcus* were obtained from the Culture Collection of Baltic Algae (CCBA; <https://ccba.ug.edu.pl/pages/en/home.php>). Pre-cultures of picocyanobacteria strains were maintained in Tissue Culture Flasks (VWR International, Cat. No. 10062-872, PA, USA) and were transferred to fresh f/2 media (Guillard 1975) at salinity of 8 PSU (which corresponds to their natural habitat) every two weeks, under a photoperiod of 12 h and Photosynthetically Active Radiation (PAR) of 10 µmol photons m−2s−1 supplied from cool white fluorescent tubes, at 22℃.

Experimental cultures of each strain were grown in 8 x 80 mL round bottom cylindrical glass tubes in a Multi-Cultivator MC 1000-OD (Photon Systems Instruments, Drásov, Czech Republic). Each culture tube contained 75 mL of f/2 medium inoculated with 5 mL of growing pre-culture, to achieve exponential growth from the beginning of the experiment, with little to no lag phase upon inoculation. Culture tubes were inoculated in the afternoon while the photoregime of a sinuisoidal photoperiod commenced the following morning such that peak PAR occurred at noon each day.

Cultures grew at 22℃, with photoperiods of 8, 12, 16, or 24 h, with peak PAR of 30, 90, 180, 300, 600, or 900 µmol photons m−2s−1 independently supplied to each culture tube from white LED lamps. To approximate diel cycles, the photoperiods of 8 – 16 h were applied in a sinuisoidal shape, while the 24-hour photoperiod was applied continuously in a square shape. The area under the sinuisoidal curve is 1/2 the area under a square of equal width, therefore at equivalent peak PAR the 24 h square photoperiod cultures received 4 times the diel photon doses of the 12 h sinuisoidal photoperiod cultures.

Culture tubes were closed with a silicone inert silicone stopper perforated by an aeration input tube extending to the bottom of the culture tube, and a pressure outlet tube. Aeration with a total air flow rate of around ~ 140 mL min−1 tube−1 through a 0.2µm filter ensured mixing and provided sufficient air/CO2 supply to cultures through the entire culture volume. The pH of tested cultures did not fluctuate fiercely during the experiment and remained at approximately 8 – 9. Light, temperature, optical density, and aeration gas of the Multi-Cultivator system were monitored and controlled via the Photobioreactor Control Software (Photon Systems Instruments, Drásov, Czech Republic).

## DNA extractions and phylogenetic analysis

Samples for total genomic DNA were collected by harvesting 10 mL of each culture and centrifuging for 8 minutes at 8,000 x. DNA was extracted using the FastDNA™ SPIN Kit for Soil (MP Biomedicals) with Matrix E columns following manufacturer instructions with the addition of an incubation with proteinase-K (1% final concentration) at 55°C for one hour. DNA concentration was measured using an Invitrogen Qubit 2.0 fluorometer (Thermo Fisher Scientific Inc.) and purity was assessed using a Thermo Scientific™ NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc.).

The phylogenetic placement of CCBA strains within cluster 5 picocyanobacteria was explored by amplifying and sequencing a fragment of the 16S rRNA gene using universal primers 27F and 1492R (Lane 1991). The amplification reactions were carried out in 25 µL final volume reactions containing 10 ng of template DNA, 0.5 μM of each primer and commercial PCR mix (Phusion High-Fidelity PCR Master Mix, Thermo Scientific) on a T100™ Thermal Cycler (Bio-Rad Laboratories, USA). The cycling conditions were: initial denaturation at 98℃ for 5 min, followed by 30 cycles at 98℃ for 40 s, annealing temperature specific for each assay for 40 s, extension at 72℃ for 1 min; and a final extension step at 72°C for 10 min PCR products were sent for Sanger sequencing (Macrogen Europe, Amsterdam, the Netherlands). 16S rRNA gene sequences were aligned with MAFFT v. 7.5 using the G-INS-I algorithm (with default parameters) (Katoh et al. 2019). Phylogenetic trees were created using IQ-TREE v. 1.6.12 with default parameters (Hoang et al. 2018; Minh et al. 2020), using GTR+F+I+I+R3 model determined by ModelFinder (Kalyaanamoorthy et al. 2017). Bootstrap values were calculated with 1000 replicates (Hoang et al. 2018). Sequences used in this study are available in GenBank under the accession numbers PP034393, PP034394, PP034396 and PP034403. 16S rRNA gene sequences were used to assign taxonomy at the family level. Phylogenetic tree of tested PC-rich and PE-rich strains is presented in Fig. 1S (Supporting Information).

## Growth curves and chlorophyll-specific exponential growth rates

Picocyanobacterial growth was monitored every 5 minutes by automatically recording OD680, OD720, and ΔOD (ΔOD = OD680 – OD720) for 14 days, independently for each culture tube. The exceptions were experiments conducted with a photoperiod of 24 h and light of 600 or 900 µmol photons m−2s−1, which lasted 7 days (Fig S2 in Supporting Information). The chlorophyll-specific exponential growth rates (µ) were determined by fitting logistic growth curves using a modified Levenberg-Marquardt fitting algorithm (Elzhov et al. 2023) to plots of the chlorophyll *a* proxy of ΔOD vs. elapsed time for each combination of strain, photoperiod, and peak PAR (Fig. S3 in Supporting Information).

To summarize the growth responses of the four picocyanobacterial strains we used a Generalized Additive Model (GAM) (Wood 2017) was applied to the relation of chlorophyll-specific µ, d-1 to photoperiod and PAR level. The R package *mgcv* (Wood 2017) was used to model the growth rate with smoothing terms and indicate the 90, 50 and 10% quantiles for growth rate across the levels of factors. Only growth rate estimates for which the amplitude of standard error was smaller than 50% of the fitted growth rate were included in the GAM. We visually compared the GAM contours to isoclines of equal cumulative diel PAR (µmol photons m−2d−1).

The 1st derivative of OD680 taken over 1 h increments was computed using *xts*: eXtensible Time Series (Ryan et al. 2024) and *signal*: Signal Processing (Ligges et al. 2024) R packages. The time when the cultures reached their maximum absolute hourly growth (tMaxAHG) of the 1st derivative of OD680 was taken as the time of transition from exponential to pre-stationary growth phases (Fig. 1).



**Fig.** 1: Example of a growth curve (tracked as OD720, OD680, or ΔOD; red solid lines, left y-axis) of PE-rich culture of *Synechococcus* sp. (048) vs. elapsed time (d, x-axis). 1st derivative of OD680 taken over 1 h increments (black solid line, right y-axis); solid blue line shows logistic fits of chlorophyll proxy OD680 – OD720 (ΔOD) vs. elapsed time. The vertical red dot dash line represents the time when the culture reached the maximum of the 1st derivative of OD680, or maximum absolute hourly growth (tMaxAHG), taken as the time of transition from exponential to pre-stationary growth phases.

## Picocyanobacteria cell counts

Picocyanobacterial cells (cell mL−1) were counted using an ImageXpress Pico Digital microscope equipped with CMOS camera and LED+ image autofocus (ImageXpress Pico Automated Cell Imaging System, Molecular Devices, LLC., CA, USA). Culture samples were preserved with 4% glutaraldehyde and kept at -80°C until the measurements. Samples (V = 10 µL) were transferred to Tissue Culture (TC)-treated surface, flat bottom black 96-well plates (Corning® Falcon® Microplate, MilliporeSigma, Merck, Darmstadt, Germany) containing 200 µL of f/2 media and centrifuged using a Beckman J-20 centrifuge with a swing bucket JS-4.3 rotor at 4500 rpm (Beckman Coulter, Brea, California, United States). Cells were imaged with the Cy5 channels (Excitation: 630/40 nm; Emission: 695/45 nm; Dichroic: 655 nm) using selectable confocal geometries, which allowed us to distinguish cyanobacterial cells from any co-occurring heterotrophic bacteria, and counted using a 63x objective in fluorescence imaging modes. Quantitative analysis on images acquired from automated microscopy obtained from 96-well microplates was performed using CellReporterXpress Image Acquisition and Analysis Software. The actual cell number was calculated based on the dilution factor and selected area count in each well.

## Whole-cell absorbance spectra

Absorbance measurements on intact cells in suspension were conducted in an integrating cavity upgrade spectrophotometer (CLARiTY 17 UV/Vis/NIR, On-Line Instrument Systems, Inc., Bogart, GA, USA). 8 mL of f/2 medium were added to both the sample and reference observation cavities of the spectrophotometer. After recording a baseline from 375 to 710 nm, 1 mL was withdrawn from the sample cavity and replaced with 1 mL of picocyanobacteria cell suspension. The pathlength corrected absorbance per cm was performed by determining the Jávorfi coefficients (Jávorfi et al. 2006) as described in the equipment manual.

## Photosynthetically Usable Radiation (PUR)

Using whole-cell absorbance spectra of *Synechococcus* sp. cultures (Fig. 2), we estimated Photosynthetically Usable Radiation (PUR; µE = µmol photons m−2s−1) according to (Morel 1978). We normalized the obtained whole-cell Absorbances (A) and the Emission spectra of the white LED lamps (Em) from 400 nm to 700 nm to a reference wavelength of 440 nm. PUR is then the ratio of the sum of Absorbance Normalized to 440 nm (NormA440) multiplied by the sum of Emission spectra Normalized to 440 nm (NormEm440) to the sum of the Emission spectra Normalized to 440 nm (NormEm440), multiplied by the PAR (Eq. (1)).



**Fig.** 2: Whole-cell absorbance spectra of PC-rich (solid green lines) or PE-rich (dashed red lines) cultures of *Synechococcus* sp. Representative absorbance spectra, normalized to 440 nm (NormA440), were measured from the exponential or pre-stationary phases of growth, together with emission spectra of the white LED lamp used for PAR, normalized to emission at 440 nm (NormEm440, light gray area), in this example PAR was 300 µmol photons m−2s−1. Estimated Photosynthetically Usable Radiation (PUR) is shown as a darker green area for the PC-rich strain and a darker red area for the PE-rich strain, with PUR given for each culture (µE = µmol photons m−2s−1). Peaks characteristic of known pigments are labeled; Chl *a*, chlorophyll *a*; PC, phycocyanin; PE, phycoerythrin; PUB, phycourobilin; Car, carotenoids.

## Cumulative diel PAR and PUR

Based on the length and shape of the photoperiod (sinuisoidal wave for photoperiods of 8, 12, 16 h; square for photoperiod of 24 h) and the peak PAR (µE = µmol photons m−2s−1), we estimated the value of the cumulative diel PAR (µmol photons m−2d−1). For sinuisoidal photoperiods we used Eq. (2); for the continuous 24 h photoperiod we used Eq. (3). Cumulative diel PUR was estimated similarly after estimation of peak PUR from peak PAR.

## Pigment content

Chlorophyll *a* (Chl *a*) (µg mL−1) was measured using Trilogy Laboratory Fluorometer (Turner Designs, Inc., CA, USA) equipped with Chlorophyll In-Vivo Module, previously calibrated using 20 mL ampoules with known Chl *a* concentrations in 3:2 90% acetone:DMSO solution. Quantitative analysis of Chl *a* was obtained after adding 50 µL of culture and 2 mL of a 90% acetone:DMSO solution in a 3:2 ratio.

We also estimated the pigment content (µg mL−1): chlorophyll *a* (Chl *a*), carotenoids (Car), phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC) in *Synechococcus* sp. cultures over time using previously determined linear correlations between pigment content obtained by extraction (Strickland and Parsons 1972; Bennett and Bogorad 1973) and absorbance values of individual pigment peaks (Car; 480, PE; 565, PC; 620, APC; 650, and Chl *a*; 665 nm) obtained from the whole-cell absorbance spectra using integrating cavity upgrade spectrophotometer (CLARiTY 17 UV/Vis/NIR, On-Line Instrument Systems, Inc., Bogart, GA, USA) (Tab. S1, Supporting Information). The sum of phycobiliproteins (PE, PC, APC protein) to Chl *a* ratio (µg:µg) for individual strains was also calculated.

## PSII effective absorption cross section of PSII and electron flux

We harvested 2 mL of cultures for photophysiological characterizations repeatedly across the growth trajectories. We used Fast Repetition Rate fluorometry (Kolber et al. 1998) (FRRf, Solisense, USA), with a lab built temperature control jacket (22℃), to apply series of flashlets to drive saturation induction/relaxation trajectories, fit using the onboard Solisense LIFT software (Falkowski and Kolber 1993; Kolber et al. 1998). From the model fits we took the initial fluorescence before induction (*F*O, *F*O′, or *F*S, depending upon the level of actinic light and step in the light response curve); the maximum fluorescence (*F*M or *F*M′) once Photosystem II (PSII) was driven to closure; and the effective absorption cross section for PSII photochemistry (σPSII or σPSII′; nm2 quanta−1) (Tortell et al. 2021). We used a double tap protocol (Xu et al. 2017), where FRRf induction/relaxation trajectories were collected during a rapid light curve sequence increasing in steps of 10 s at 0, 20, 40, 80, 160, and 320 µmol photons m−2s−1 PAR, delivered from LED emitters centred at 445, preferentially exciting chlorophyll, or 590 nm, preferentially exciting phycobiliproteins. Flash Power for 445 nm excitation was 60000 µmol photons m−2s−1 PAR, while for 590 nm excitation power was 14000 µmol photons m−2s−1, calibrated using a quantum sensor (LI-250, LI-COR, Inc.). We applied 1 s darkness between sequential light steps, to allow re-opening of PSII. FRRf excitation flashlets were applied at the same wavebands, 445 or 590 nm, as the actinic light steps.

We calculated (Eq. (4)) an uncalibrated fluorescence based estimator for volumetric electron transport, *JV*PSII, (k × e− L−1 s−1) under both 445 and 590 nm excitation bands (Oxborough et al. 2012; Boatman et al. 2019; Tortell et al. 2021).

where σPSII′ is effective absorption cross section for PSII photochemistry under the relevant actinic PAR step (nm2 quanta−1); qP is an estimate of the fraction of PSII open for photochemistry estimated according to Oxborough and Baker (1997); I is the applied PAR (µmol photons m−2s−1); *F*O is the minimum fluorescence from a given sample and excitation bandwidth (relative fluorescence) and σPSII is the maximum effective absorption cross section for PSII photochemistry from a given sample and excitation bandwidth (nm2 quanta−1). We compared several other algorithms for *JV*PSII (Tortell et al. 2021) and found similar results.

We calibrated the *JV*PSII estimator to absolute rates of electron transport (Eq. (5)) using parallel measures of oxygen evolution (µmol O2 L−1 s−1), captured simultaneously with the FRRf measures, below light saturation of electron transport, using a FireSting robust oxygen probe (PyroScience, Germany) inserted in the cuvette for select Rapid Light Curve (RLC) runs (Fig. 3). For the blue LED (Ex445nm) excitation we used a calibration slope of 108832, while for orange LED (Ex590nm) excitation we used a calibration slope of 254327



**Fig.** 3: Single turnover (ST) fluorescence induction by Fast Repetition Rate fluorometry (FRRf). (**A**) Examples of fluorescence yield vs. elapsed time (µs) for PE-rich culture of *Synechococcus* sp. (048) in the dark (dark-relaxed; 0 µmol photons m−2s−1) and under actinic PAR (in this example 80 µmol photons m−2s−1) using blue LED (Ex445nm; open blue circles) or orange (Ex590nm; open orange diamonds) excitation. The ST technique delivers a series of flashlets for non-intrusive, repeated monitoring of chlorophyll fluorescence parameters (including *F*O, *F*′, *F*M, *F*M′, τ1-τ3, τ1′-τ3′, σPSII, and σPSII′). (**B**) Linear regressions of uncalibrated PSII electron flux (*JV*PSII) vs. e− L−1 s−1 derived from simultaneously measured oxygen evolution Light Response Curves (LRC) under blue LED (Ex445nm; open blue circles) or orange (Ex590nm; open orange diamonds) excitation. (**C**) Rapid Light Curve (RLC), fit with a three parameter model (Harrison and Platt 1986), for PSII electron flux (*JV*PSII; µmol e− µmol Chl *a*−1 s−1) vs. actinic PAR measured under blue LED (Ex445nm; open blue circles) or orange (Ex590nm; open orange diamonds) excitation.

## Statistical analysis

Analysis of results was conducted using R version 4.3.0 (R Core Team 2023) running under RStudio (Posit team 2022). To determine significant differences between measurement sets the “stats” v. 3.6.2 R standard package for statistical functions including the *lm()* function for linear regression, *aov()* function for ANOVA, and *t.test()* function for *t*-test. The *SSasymp(*) function (Self-Starting Nls Asymptotic Regression Model) was used to perform a single phase exponential decay fit model and to estimate exponential decay parameters (y0, the starting value; yf, the value at infinite x axis; λ, exponential decay constant) (Serway et al. 2004). We also used the *nlsLM()* function (Elzhov et al. 2023) to fit a three parameter model (α, initial slope of curve; *β*, reflecting the photoinhibition process; *P*max, the maximum rate of growth curve) proposed by Harrison and Platt (1986).

We performed three-way factorial ANOVA to determine whether peak PAR, photoperiod, strain, and their interactions, significantly influence the chlorophyll-specific exponential growth rate (µ; d−1), estimated from logistic fits of chlorophyll proxy OD680 – OD720 vs. cumulative diel PUR (Table S2).

To examine statistical differences between fits of light responses, we performed one-way ANOVA of the three parameter model (Harrison and Platt 1986) fit to pooled data for each taxa, compared to separate fits for each different photoperiods (8, 12, 16, or 24); or to separate fits for each different peak PAR (30, 90, 180, 300, 600 together with 900). These comparisons were run for chlorophyll-specific exponential growth rate vs. cumulative diel PUR (Table S3, S4); vs. cumulative diel PAR (Table S5, S6) or vs. PSII electron flux (*JV*PSII; µmol e− µmol Chl *a*−1 d−1; Table S7, S8). One-way ANOVA was also used to examine statistical differences between single phase exponential decay fits of pooled data across different strains for a given phase of growth and across different phase of growth for a given strain for PUR/PAR ratio (Table S9); Phycobiliprotein to Chl *a* ratio (Table S10); or effective absorption cross section of PSII (σPSII′; nm2 quanta−1) measured under diel peak PAR growth light under Ex590nm (orange) excitation in relation to the cumulative diel PAR (µmol photons m−2d−1) (Table S11).

We used *t*-tests of linear fits to compare pooled data across different strains for a given phase of growth, and across different phases of growth, for a given strain, for effective absorption cross section of PSII (σPSIIʹ; nm2 quanta−1) measured under diel peak PAR growth light under Ex445nm (blue) excitation vs. the cumulative diel PAR (µmol photons m−2d−1; Table S12); or vs. the Phycobiliprotein to Chl *a* ratio (Table S13). The same *t*-test analyses were performed for effective absorption cross section of PSII (σPSII′ or σPSII; nm2 quanta−1) measured under Ex590nm (orange) excitation vs. the Phycobiliprotein to Chl *a* ratio (Table S14, S15).

Statistical differences for all analyses were determined at significance level α = 0.05. The manuscript was prepared as a Rmarkdown document (Handel 2020) with figures plotted using the ggplot2 (Wickham 2016) and the patchwork (Pedersen 2024) packages. All metadata, data and code is available on GitHub (<https://github.com/FundyPhytoPhys/BalticPhotoperiod>).

# Results

## Chlorophyll-specific exponential growth rate

We used logistic curve fits (Fig. S3) to determine chlorophyll-specific exponential growth rates (μ; d−1), for two PhycoCyanin(PC)-rich cultures (056, 077) and two PhycoErythrin(PE)-rich cultures (048, 127) of *Synechococcus* sp. originating from the Baltic Sea grown at 30, 90, 180, 300, 600, or 900 peak PAR µmol photons m−2s−1 (µE); and photoperiods of 8, 12, 16, or 24 h. Three-way factorial ANOVA showed that peak PAR, photoperiod, strain, and their interactions, significantly affected μ (ANOVA, *p* < 0.05 for all; Table S2). All tested strains, except PE-rich\_048, grew even under peak PAR 900 µmol photons m−2s−1 and 24 h photoperiod. The highest growth rate was recorded for *Synechococcus* sp. PE-rich\_127 (μ = 4.5 d−1; 3.7 h doubling time) and PC-rich\_056 (μ = 3.4 d−1; 4.9 h doubling time) at 180 µmol photons m−2s−1 peak PAR and photoperiod of 24 h.

The GAM model in Fig. 4 summarizes the growth responses of the PC-rich and PE-rich picocyanobacteria to peak PAR and photoperiod. PC-rich\_056 *Synechococcus* sp. showed highest growth rates under a photoperiod of 24 h, across a wide range of peak PAR indicated by the contour line labeled 1.45 d−1, representing the 90th percentile of achieved growth rates for the strain. On the other hand, the other tested PC-rich strain (077) showed highest growth rates in the range of photoperiod 16-24 h and peak PAR between 300 – 700 µmol photons m−2s−1, indicated by the 1.81 d−1 contour line again representing the 90th percentile of maximum achieved growth rates for the strain. For both PC-rich strains, growth was slowest under 30 µmol photons m−2s−1 and a photoperiod of 8 h.

Both PE-rich strains achieved fastest growth rates above peak PAR of ~300 µmol photons m−2s−1, under the longest photoperiod of 24 h, indicated by the 1.97 d−1 for PE-rich\_048, and 2.34 d−1 for PE-rich\_127, contour lines. For the PE-rich strains growth decreased with decreasing photoperiod and decreasing peak PAR. Moreover, PE-rich strains showed photoinhibition of growth at peak PAR of 900 µmol photons m−2s−1 and photoperiods of 16- 24 h. The growth rate contours for PC-rich and PE-rich *Synechococcus* sp. did not generally follow isoclines of cumulative diel photon dose (µmol photons m−2d−1, dashed lines), showing that photoperiod, and peak PAR influenced growth rates beyond cumulative diel photon dose.



**Fig.** 4: A contour plot of a Generalized Additive Model (GAM) of chlorophyll-specific growth rates (d−1) for two PhycoCyanin(PC)-rich cultures: (**A**) 056, (**B**) 077 and two PhycoErythrin(PE)-rich cultures: (**C**) 048, (**D**) 127 of *Synechococcus* sp. originating from the Baltic Sea. Cultures were grown at 30, 90, 180, 300, 600, or 900 peak PAR µmol photons m−2s−1; and photoperiods of 8, 12, 16, or 24 h. Legends show colour gradients of growth rate (µ; d−1) from no growth (white) to 3.0 d−1 (dark green for PC-rich\_056, light green for PC-rich\_077, light red for PE-rich\_048 or dark red for PE-rich\_127 strains). Labeled contour lines indicate the 90%, 50%, and 10% quantiles for achieved growth rate. Dotted lines show isoclines of cumulative diel photon dose (µmol photons m−2d−1).

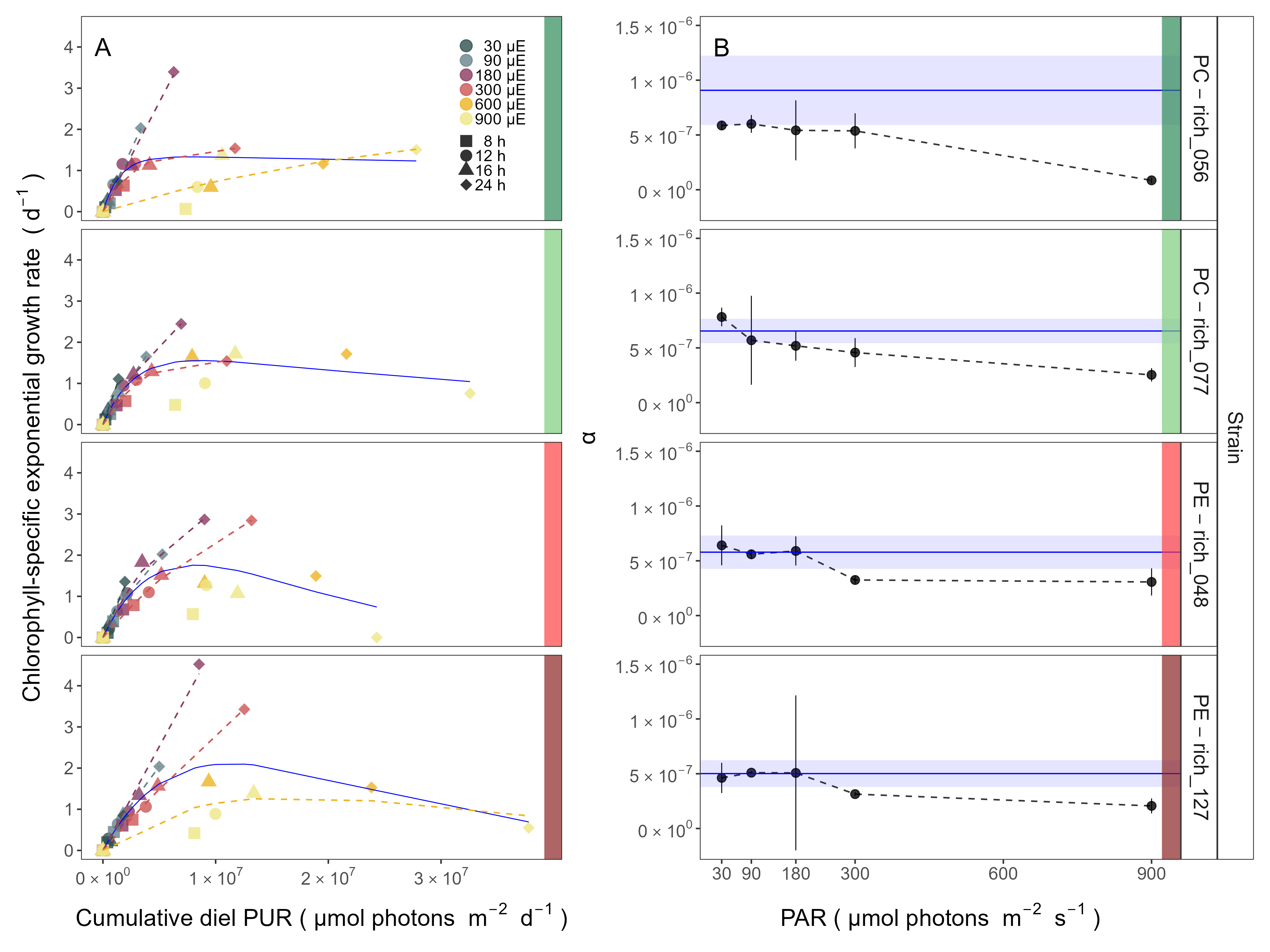
A three parameter light response model fit (Harrison and Platt 1986) of chlorophyll-specific exponential growth rates vs. cumulative diel PUR dose for two PC-rich and two PE-rich cultures of *Synechococcus* sp. showed significant differences between model fits of the pooled data vs. fits for all tested photoperiods (8, 12, 16, or 24 h; ANOVA, *p* < 0.05; Fig. 5A, Table S3). The alpha parameters of the initial rise of growth rate (α) vs. cumulative diel PUR, estimated from data pooled for each photoperiod increased with increasing photoperiod for all strains. The highest increase (>2-fold) of α with increasing photoperiod was recorded for PC-rich\_056 (Fig. 5B).



**Fig.** 5: (**A**) Chlorophyll-specific exponential growth rates (d−1) vs. cumulative diel Photosynthetically Usable Radiation (PUR, µmol photons m−2d−1). Growth rates (± SE falling within symbols) were estimated from logistic fits of chlorophyll proxy OD680 – OD720 (ΔOD) vs. elapsed time (Fig. 1, Fig. S3), for two PhycoCyanin(PC)-rich cultures (056, 077) and two PhycoErythrin(PE)-rich cultures (048, 127) of *Synechococcus* sp. originating from the Baltic Sea. Cultures were grown at 30 (dark gray), 90 (light gray), 180 (purple), 300 (red), 600 (orange), or 900 (yellow) peak PAR µmol photons m−2s−1 (µE); and photoperiods of 8 (square), 12 (circle), 16 (triangle), or 24 (diamond) h. Solid blue line shows a fit of the pooled growth rates through photoperiods for each strain, with a three parameter model (Harrison and Platt 1986). We also fit the same model separately for 8 (dotted line), 12 (long dash line), 16 (dashed line), or 24 (two dash line) h photoperiods, since for all strains they were each significantly different (ANOVA, *p* < 0.05) from the fit of pooled data. (**B**) Alpha parameters of the initial rise of growth rate (α) vs. cumulative diel Photosynthetically Usable Radiation (PUR), estimated from data pooled for each photoperiod (points (± SE) connected by dashed lines), and estimated for all data across photoperiods (solid blue horizontal line ± SE), for each strain.

Strains also showed distinct growth rate responses to cumulative diel PUR, depending upon peak PAR (Fig. 6A, Table S4), that differ from a single light response model fit to the pooled data across all peak PAR from a strain. Exceptions were observed in the strains PC-rich\_077 and PE-rich\_048 with the peak PAR of 600 or 900 µmol photons m−2s−1, which were not significantly different from the pooled data model. A caveat to these findings is that cumulative diel photon dose is a product of photoperiod and PAR, so the highest levels of cumulative PUR dose are only achieved under the 600 or 900 µmol photons m−2s−1. The alpha parameters of the initial rise of growth rate (α) vs. cumulative diel PUR, estimated from data pooled for each peak PAR decreased across peak PAR for all tested strains. Here, PC-rich strains showed a steady decrease of α parameter with increasing peak PAR whereas PE-rich strains showed a plateau in the decline in the α parameter at 300 µmol photons m−2s−1 and above (Fig. 6B).

Growth rate saturated under increasing cumulative diel PUR for all strains, however, the achieved estimates of µmax varied depending upon photoperiod and peak diel PAR. Growth rates vs. cumulative diel PAR relationships, estimated for exponential phase cultures, followed similar patterns (Fig. S4, S5 and Table S5, S6 in Supporting Information).



**Fig.** 6: (**A**) Chlorophyll-specific exponential growth rates (d−1) vs. cumulative diel Photosynthetically Usable Radiation (PUR, µmol photons m−2d−1). Growth rates (± SE falling within symbols) were estimated from logistic fits of chlorophyll proxy OD680 – OD720 (ΔOD) vs. elapsed time (Fig. 1, Fig. S3), for two PhycoCyanin(PC)-rich cultures (056, 077) and two PhycoErythrin(PE)-rich cultures (048, 127) of *Synechococcus* sp. originating from the Baltic Sea. Cultures were grown at 30 (dark gray), 90 (light gray), 180 (purple), 300 (red), 600 (orange), or 900 (yellow) peak PAR µmol photons m−2s−1 (µE); and photoperiods of 8 (square), 12 (circle), 16 (triangle), or 24 (diamond) h. Solid blue line shows a fit of the pooled growth rates through peak PAR for each strain, with a three parameter model (Harrison and Platt, 1986). We also fit the same model separately for 30 (dark gray), 90 (light gray), 180 (purple), 300 (red), 600 together with 900 (orange) peak PAR µmol photons m−2s−1, only when they were each significantly different (ANOVA, *p* < 0.05) from the fit of pooled data. (**B**) Alpha parameters of the initial rise of growth rate (α) vs. cumulative diel Photosynthetically Usable Radiation (PUR), estimated from data pooled for each peak PAR (points (± SE) connected by dashed lines), and estimated for all data across all peak PAR, for each strain (solid blue horizontal line ± SE).

## PUR/PAR ratio vs. cumulative diel PAR

The PUR/PAR ratio is an index of the efficacy of light capture for a culture under a given growth condition; showing the fraction of PAR that can be captured by the absorbance of the cells (Fig. 7). For the two PC-rich and, particularly, for the two PE-rich cultures of *Synechococcus* sp. PUR/PAR decayed exponentially to a plateau, with increasing cumulative diel PAR, when pooling PUR/PAR data across different combinations of photoperiod and peak PAR. Although all strains followed a similar trend, the single phase exponential decay model fit parameters varied significantly among strains, during their exponential phase of growth (ANOVA, *p* < 0.05), except the model fits from PE-rich\_048 and PE-rich\_127 (ANOVA, *p* > 0.05; Table S9). Moreover, the PUR/PAR ratio was higher in the PE-rich strains under low cumulative diel photon dose during their exponential phase of growth (y0 greater or equal to 0.9), but decayed towards a plateau close to the PC-rich strains as cumulative diel photon dose increases (yf = 0.5). On the other hand, the single phase exponential decay model fits did not differ significantly among strains, during their pre-stationary phase of growth (ANOVA, *p* > 0.05; Table S9). During this phase, response of PUR/PAR ratio to increasing cumulative diel PAR exhibits damping, maintaining a consistent trend across all strains within the yf range of 0.4 to 0.5, with the exception of the PE-rich\_048 strain. We also find that model fits from different phases of growth differed within a given strain, with the exception of PC-rich\_056 (ANOVA; *p* < 0.05, Table S9). A similar decay trend was observed for Phycobiliprotein to Chl *a* ratio (µg:µg) across cumulative diel PAR (Fig. S6).



**Fig.** 7: Changes in PUR/PAR ratio vs. cumulative diel PAR (µmol photons m−2d−1). PUR/PAR ratio was estimated for two PhycoCyanin(PC)-rich cultures (056, 077) and two PhycoErythrin(PE)-rich cultures (048, 127) of *Synechococcus* sp. originating from the Baltic Sea. Cultures were grown at 30 (dark gray), 90 (light gray), 180 (purple), 300 (red), 600 (orange), or 900 (yellow) peak PAR µmol photons m−2s−1 (µE); and photoperiods of 8 (square), 12 (circle), 16 (triangle), or 24 (diamond) h. Figure presents data (smaller symbols) and means (bigger symbols) from exponential or pre-stationary phase of growth. Blue solid line shows single phase exponential decay fit for data from each strain and growth phase, with fit parameters presented. Different lowercase letters indicate statistically significant differences between the fit models for different strains within a given phase of growth. Different uppercase letters indicate statistically significant differences between the fit models for different phases of growth within a given strain (ANOVA; *p* < 0.05).

## Effective absorption cross section of PSII of picocyanobacteria

The effective absorption cross section of PSII (σPSIIʹ, nm2 quanta−1), was estimated using FRRf induction curves using Ex590nm (orange) excitation, for two PC-rich (056, 077) and two PE-rich (048, 127) cultures of *Synechococcus* sp. grown at 30, 90, 180, 300, 600, or 900 peak PAR µmol photons m−2s−1 (µE); and photoperiods of 8, 12, 16, or 24 h (Fig. 8). The σPSIIʹ measured under diel peak PAR growth light under Ex445nm (blue) excitation vs. cumulative diel photon dose is shown in Supporting Information (Fig. S7, Table S12).

All strains showed consistent patterns of sharp, exponential decay of effective absorption cross section for PSII photochemistry vs. cumulative diel photon doses, across different combinations of photoperiod and peak PAR (Fig. 8A). Although all strains showed this response pattern, the exponential decay fits differed significantly among two PC-rich strains and PE-rich\_048 strains during their exponential phase of growth (ANOVA, *p* < 0.05; Table S11). PE-rich strains showed higher σPSIIʹ under low cumulative diel photon dose (y0 about 0.8 and yf about 4) than did PC-rich strains. During pre-stationary phase this response dampens in the PC-rich strains but persists in the PE-rich strains (Table S11). σPSIIʹ for the PE-rich strains during pre-stationary phase of growth still remain higher (yf between 2.3 – 3.0) than in the PC-rich strains (yf between 1.4 – 1.7) even as cumulative diel photon dose increases. Model fits from different phases of growth differed within a given strain, with the exception of PE-rich\_048 (ANOVA; *p* < 0.05, Table S11).



**Fig.** 8: (**A**) Effective absorption cross section of PSII (σPSIIʹ; nm2 quanta−1) measured under diel peak PAR growth light vs. cumulative diel PAR (µmol photons m−2d−1); blue solid line shows single phase exponential decay fit for data from each strain and growth phase. (**B**) Changes of σPSIIʹ measured under diel peak PAR growth light vs. the ratio of sum of µg phycobilins (PE, PC, APC protein, Phycobiliprotein) to µg Chl *a*; blue solid line shows linear model fit for data from each strain and growth phase. σPSIIʹ was estimated using FRRf induction curves with excitation of phycobilisomes (Ex590nm, orange), for two PhycoCyanin(PC)-rich cultures (056, 077) and two PhycoErythrin(PE)-rich cultures (048, 127) of *Synechococcus* sp. originating from the Baltic Sea. Cultures were grown at 30 (dark gray), 90 (light gray), 180 (purple), 300 (red), 600 (orange), or 900 (yellow) peak PAR µmol photons m−2s−1 (µE); and photoperiods of 8 (square), 12 (circle), 16 (triangle), or 24 (diamond) h. Figure presents data (smaller symbols) and means (bigger symbols) from exponential or pre-stationary phase of growth. Different lowercase letters indicate statistically significant differences between the fit models for different strains within a given phase of growth. Different uppercase letters indicate statistically significant differences between the fit models for different phases of growth within a given strain (*p* < 0.05).

Effective absorption cross section of PSII (σPSIIʹ; nm2 quanta−1), measured under diel peak PAR growth light with Ex590nm (orange) excitation, varies with Phycobiliprotein to Chl *a* ratio (Fig. 8B). σPSIIʹ excited through phycobilisome absorbance at Ex590nm shows positive linear correlations with the Phycobiliprotein to Chl *a* ratio, although strains in exponential growth show significant scatter around this positive relation, likely related to regulatory control of σPSIIʹ under different measurement PAR, beyond pigment composition. Under pre-stationary phase the relationship between σPSIIʹ and Phycobiliprotein to Chl *a* ratio was more consistent, suggesting increased reliance upon compositional regulation to control light delivery to PSII, as opposed to shorter-term physiological regulation under changing light. The linear fits of σPSIIʹ vs. Phycobiliprotein to Chl *a* ratio also vary significantly between PC-rich\_077 and two PE-rich strains during their exponential phase of growth. During pre-stationary phase we noted significant differences between two PC-rich strains and PE-rich\_048. Moreover, significant differences between the fit models for varying phases of growth were noted for PC-rich strains 056 and 077 (*t*-test; *p* < 0.05, Table S14).

Changes in effective absorption cross section of PSII (σPSII; nm2 quanta−1) measured in the dark with Ex590nm (orange) excitation vs. Phycobiliprotein to Chl *a* ratio (Fig. S8A, Table S15) and σPSIIʹ measured under diel peak PAR growth light under Ex445nm (blue) excitation vs. Phycobiliprotein to Chl *a* ratio (Fig. S8B and Table S13) are shown in Supporting Information.

## Chlorophyll-specific exponential growth rates vs. cumulative diel PSII electron flux

Chlorophyll-specific exponential growth rates (d−1), within each strain, show fairly consistent saturating responses to increasing cumulative diel PSII electron flux (*JV*PSII; µmol e− µmol Chl *a*−1 d−1) estimated under diel peak PAR growth light, and estimated using FRRf induction curves with excitation of chlorophyll (Ex445nm, blue), although photoperiod (Fig. 9A, Table S7) and peak PAR (Fig. S9, Table S8) retained a secondary influence on achieved growth responses for some growth conditions.

A three parameter model fit of (Harrison and Platt 1986) vs. cumulative diel PSII electron flux (*JV*PSII; µmol e− µmol Chl *a*−1 d−1) for two PC-rich and two PE-rich cultures of *Synechococcus* sp. showed no significant differences between fits of the pooled data vs. fits for different photoperiods (8, 12, 16, or 24 h; ANOVA, *p* < 0.05), with exception of 8 and 24 h photoperiod for PC-rich\_056 and 8 h photoperiod for PE-rich\_127 strains (ANOVA, *p* > 0.05; Table S7).

Alpha parameters of the initial rise of growth rate (α) vs. cumulative diel *JV*PSII, estimated from data pooled for each photoperiod showed an increase across increasing photoperiods for each strain except for PE-rich\_0127. The highest increase (>2-fold) of α from the lowest to the highest photoperiod was recorded for PC-rich\_077 (Fig. 9B).



**Fig.** 9: (**A**) Chlorophyll-specific exponential growth rates (d−1) vs. cumulative diel PSII electron flux (*JV*PSII; µmol e− µmol Chl *a*−1 d−1) measured under diel peak PAR growth light. Growth rates (± SE falling within symbols) were estimated from logistic fits of chlorophyll proxy OD680 - OD720 (ΔOD) vs. elapsed time (Fig. S3). PSII electron flux (*JV*PSII; µmol e− µmol Chl *a*−1 d−1) was estimated using FRRf induction curves with excitation of chlorophyll (Ex445nm, blue), for two PhycoCyanin(PC)-rich cultures (056, 077) and two PhycoErythrin(PE)-rich cultures (048, 127) of *Synechococcus* sp. originating from the Baltic Sea. Cultures were grown at 30 (dark gray), 90 (light gray), 180 (purple), 300 (red), 600 (orange), or 900 (yellow) peak PAR µmol photons m−2s−1 (µE); and photoperiods of 8 (square), 12 (circle), 16 (triangle), or 24 (diamond) h. Solid blue line shows a fit of the pooled growth rates for each strain, with a three parameter model (Harrison and Platt 1986). We also fit the same model separately for 8 (dotted line) and 24 (two dash line) h photoperiods, when they were significantly different (ANOVA, *p* < 0.05) from the fit of pooled data. (**B**) Alpha parameters of the initial rise of growth rate (α) vs. cumulative diel *JV*PSII, estimated from data pooled for each photoperiod (points (± SE) connected by dashed lines), and estimated for all data across photoperiods (horizontal line ± SE), for each strain.

# Discussion

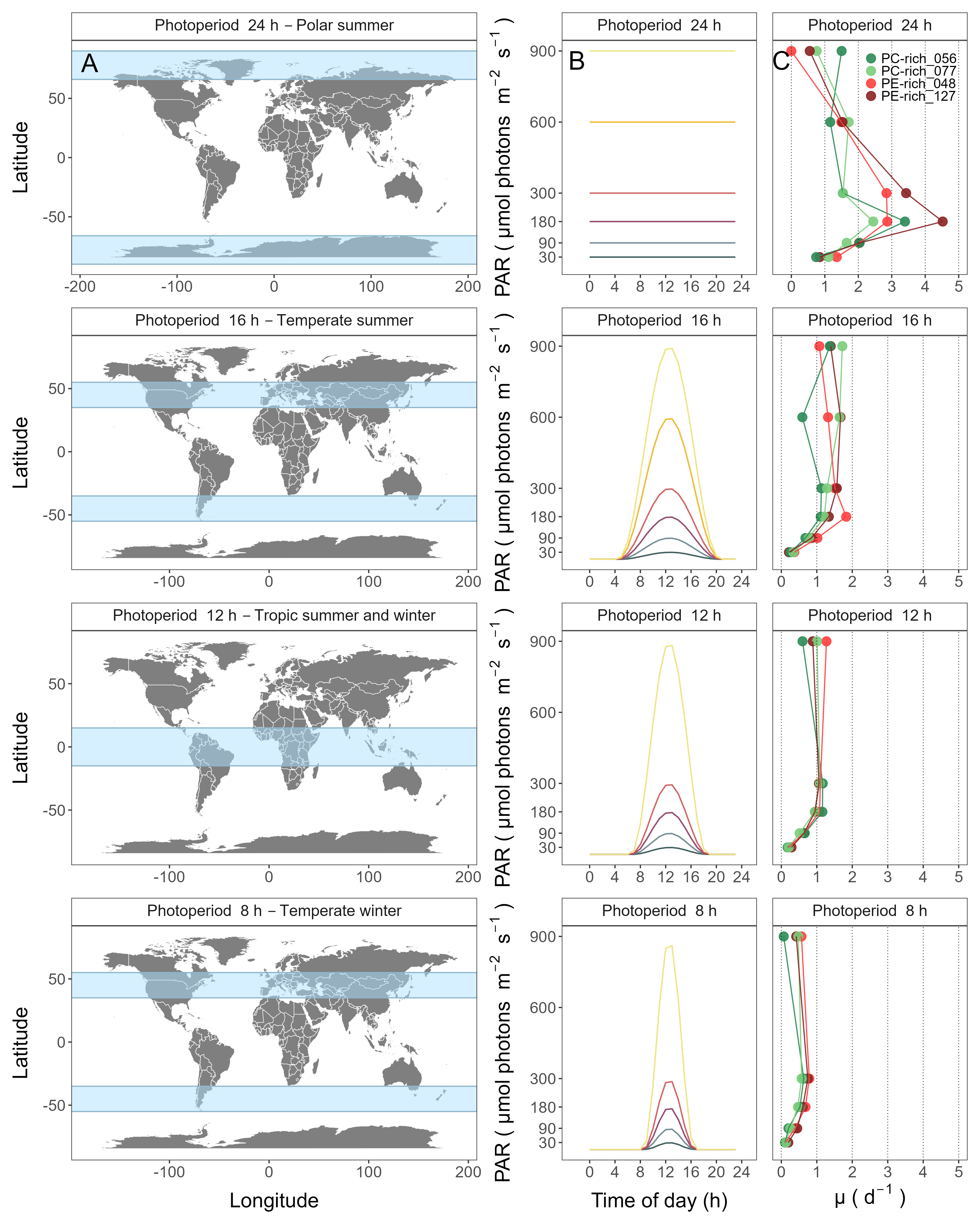
## Photic regimes - implications for picocyanobacteria growth and distribution

Light regimes, including photoperiod, and peak PAR, are major factors affecting the distribution and seasonality of phytoplankters (Erga and Heimdal 1984). Changes in photoperiod trigger acclimation responses, shaping the temporal dynamics and community structure of phytoplankton (Theus et al. 2022; Longobardi et al. 2022). Each tested picocyanobacterial strain showed influences of photoperiod upon the responses of growth rate to cumulative diel PUR (Fig. 5). To our surprise, increasing photoperiod increased the ranges of response to PAR and PUR. Both the PC-rich and, particularly, the PE-rich strains of *Synechococcus* sp. exhibited their fastest growth rates under continuous light (24 h photoperiod). Jacob-Lopes et al. (2009) showed an increase in biomass production of picocyanobacterium *Aphanothece microscopica* with increasing duration of the light period. The PC-rich thermophilic *Synechococcus* PCC 6715 (*Thermostichus lividus* PCC 6715) (Klepacz-Smółka et al. 2020) showed faster growth under a 24 h photoperiod than under 16 h photoperiod. For our tested temperate strains, there is no direct selective pressure for exploitation of a continuous 24 photoperiod (Brand and Guillard 1981), so achieving maximum growth under a 24 h photoperiod rather suggests absence of a requirement for a dark period, or absence of requirement for a regular photoperiod. Coastal strains are selected to exploit instantaneous light (Brand and Guillard 1981), of whatever duration, to cope with fluctuating light and nutrients in coastal environments (MacIntyre et al. 2000; Litchman et al. 2009), leading to a pleiotropic capacity for exploiting continuous light. This ability of both PC-rich and PE-rich coastal picocyanobacteria to exploit continuous light means they could, potentially, grow rapidly at higher latitudes, in a future warmer polar summer water.

Light level is another key driver of picocyanobacteria productivity (Pick 1991; Six et al. 2007; Aguilera et al. 2023). PE-rich and PC-rich *Synechococcus* sp. strains show distinct growth responses to cumulative diel photon dose, depending upon peak PAR (Fig. 6, S6). Chlorophyll-specific exponential growth rates of the PE-rich and PC-rich *Synechococcus* sp. strains increased with increasing light levels, to a plateau in the range of 180 – 300 µmol photons m−2s−1. Growth above 600 µmol photons m−2s−1 occurred, but the growth yield per cumulative diel photon was lower than under moderate light, particularly when combined with short 8 h or long 24 h photoperiods. Even though PE-rich *Synechococcus* sp. are more adapted to lower-light conditions deeper in the water column (Six et al. 2007), our findings show that PE-rich strains will grow under higher irradiance.

The maximum growth rate of *Synechococcus* sp. originating from the Baltic Sea, achieved by the PE-rich\_127 strain under 24 h photoperiod, and peak PAR of 180 µmol photons m−2s−1 was 4.5 d−1 (µ = 0.187 h−1) corresponding to a doubling time of 3.7 h (Fig. 5, Fig. 6); a growth rate not previously reported for marine picocyanobacteria. This growth rate of PE-rich *Synechococcus* sp. is faster than for the model freshwater cyanobacteria *Synechococcus* sp. PCC6301 (doubling time of 4.5-5 h under constant illumination and 250 µmol photons m−2s−1) noted by Sakamoto and Bryant (1999), and for model cyanobacteria *Synechocystis* sp. PCC 6803 (doubling time of 4.3 h) (van Alphen et al. 2018). The fastest growth rate as yet achieved for any phytoplankter occurs in a genetically modified green algae *Picochlorum celeri*, with a maximum of about 6.8 d−1 and ~2.5 h doubling time, in bioreactors (Krishnan et al. 2021). The Baltic *Synechococcus* sp. strains, not genetically modified, preferred 24 h photoperiod and moderate peak PAR of 180 µmol photons m−2s−1, suggesting they could, potentially, thrive in warming polar latitude waters. *Synechococcus* sp. strains already occur across geographical regions (Śliwińska-Wilczewska et al. 2018b) with different photic regimes, including polar regions (reviewed by Velichko et al. (2021)), exceeding latitude 80°S and 80°N. During the Arctic or Antarctic summer, prolonged daylight hours, coupled with nutrient-rich waters, promote the growth of genetically diverse *Synechococcus* populations (Vincent et al. 2000), contributing significantly to primary productivity. Gradinger and Lenz (1989) suggested that *Synechococcus*-type picocyanobacteria can serve as indicator organisms for the advection of warm water masses into polar regions, important in the context of monitoring upcoming climate changes.

In our study, PC-rich and PE-rich strains of *Synechococcus* showed saturation, and then photoinhibition of growth rates under increasing cumulative diel PUR, although the achieved estimates of µmax, and the onset of photoinhibition of growth, varied depending upon strain, photoperiod and peak PAR (Fig. 4). The tested strains were generally opportunistic in exploiting longer photoperiods to achieve faster µ, although PE-rich strains suffered strong photoinhibition of growth under peak PAR above 600 µmol photons m−2s−1 and 24 h photoperiod (Fig. 5, Fig. 6), suggesting the PE-rich strains are better adapted to lower light and deeper parts of the water column. The least favorable growth conditions for both PE-rich and PC-rich strains of *Synechococcus* sp. were under high light (> 600 µmol photons m−2s−1) and the shortest photoperiod (8 h), even though the cumulative diel PUR dose was equivalent to conditions where the light intensity was lower and the photoperiod was longer. Thus these Baltic picocyanobacteria are prone to photoinhibition under both the longest, and the shortest, photoperiod regimes, with flatter light responses of growth under intermediate photoperiods. Thus, in regions and periods with a longer photoperiod, both PC-rich and PE-rich *Synechococcus* sp. could become dominant species in surface waters, but could suffer under shorter photoperiods (Fig. 10). We next aim to test how different spectral bands of light, in combination with temperature and oxygen availabilities, affect the physiologies of picocyanobacteria in order to predict their occurrence throughout water columns, under the influence of expected climate changes.



**Fig.** 10: Latitudinal bands, equivalent summer or winter photoperiods, and picocyanobacterial growth responses. (**A**) Latitudinal bands corresponding to tested growth photoperiods. (**B**) Tested photoperiod and peak PAR regimes used for growth experiments. (**C**) Chlorophyll specific exponential growth rates (± SE falling within symbols) for two PhycoCyanin(PC)-rich cultures (056; dark green, 077; light green) and two PhycoErythrin(PE)-rich cultures (048; light red, 127; dark red) of *Synechococcus* sp. under tested photoperiod and peak PAR regimes.

## Photic regimes and growth phase - implications for light capture and light absorption

The Photosynthetically Usable Radiation (PUR) represents the light available for phytoplankton to photosynthesize. PUR is always smaller than PAR (PUR < PAR), and depends on the spectral composition of the PAR, versus the phytoplankton pigment composition, determining cellular spectral absorption (Morel 1978). The spatial and temporal distribution of PAR within aquatic ecosystems is influenced by solar angle, water depth, water clarity, and the presence of light-absorbing substances such as dissolved organic matter (Morel 1978, 1988) and phytoplankton cells. PUR, in turn, is also determined by pigment contents of phytoplankton cells, which changes depending upon growth conditions and the phase of growth. We find that under nutrient replete exponential growth the picocyanobacterial strains show consistent patterns of of an exponential decline in PUR/PAR ratio versus cumulative diel photon doses, across different combinations of photoperiod and peak PAR. Thus under nutrient repletion the picocyanobacteria balance pigment composition to match light conditions (Fig. 7). In addition to chlorophyll *a*, picocyanobacteria utilize phycobilins, including phycocyanin (harvesting red light at 620 nm) and phycoerythrin (harvesting yellow light at 570 nm), as accessory pigments to enhance light harvesting efficiency (Fig. 2). Adapting to low-light environments, picocyanobacteria enhance phycobilin production to compensate for limited irradiance, thereby optimizing their photosynthetic capabilities (Śliwińska-Wilczewska et al. 2018a).

The effective absorption cross section for photochemistry of PSII in the light (σPSIIʹ) comprises the probability of light capture by PSII and the quantum yield for subsequent photochemistry. PC-rich and PE-rich strains of *Synechococcus* show consistent patterns of an exponential decay to a plateau with increasing cumulative diel PAR doses, for σPSIIʹ (nm2 quanta−1, measured under diel peak PAR growth light under Ex590nm (orange) excitation), without detectable influences of photoperiod, nor of peak PAR (Fig. 8A). σPSIIʹ excited through chlorophyll absorbance at 445 nm was, in contrast, consistently small across strains and growth conditions (Fig. S9), since in cyanobacteria the number of chlorophyll serving each PSII is nearly fixed (Xu et al. 2018). σPSIIʹ excited through phycobilisome absorbance at 590 nm shows, as expected, a positive correlation with phycobilin:chlorophyll ratio. Growth under low cumulative diel PAR results in an increased phycobilin:chlorophyll ratio, as the picocyanobacteria allocate protein resources towards phycobiliprotein-mediated light capture (Beale 1994; Stadnichuk et al. 2015; Chakdar and Pabbi 2016). PC-rich and PE-rich strains of *Synechococcus* sp. in exponential growth nonetheless show significant scatter around this pattern, likely related to regulatory control of σPSIIʹ, beyond pigment composition. Under pre-stationary phase σPSIIʹ vs. Phycobiliprotein to Chl *a* ratio was better aligned, suggesting reliance upon fixed compositional regulation of phycobiliprotein content to control light delivery to PSII, as opposed to shorter-term regulation.

The phylogeny based on the 16S rRNA gene (amplicon average 1385 bp) placed the tested strains to order Synechococcales and family Synechoccaceae, within the cluster 5 picocyanobacterial lineage, in sub-cluster 5.2 together with freshwater, brackish and halotolerant strains, separated from marine sub-clusters 5.1 and 5.3 (Fig. 1S in Supporting Information). Sequences had high identity of 16S rRNA (∼100%) with strains assigned to either *Synechococcus* spp. or *Cyanobium* spp. However, it is worth emphasizing here that light capture and light absorption abilities differ significantly among tested strains. The PE-rich strains show a much higher PUR/PAR ratio under low cumulative diel photon dose during their exponential phase of growth, but decay towards a plateau and reach a similar value to the PC-rich strains as the cumulative diel photon dose increases. This means that PE-rich strains in the exponential phase of growth demonstrated higher ability to modulate light absorbance capacity whereas PC-rich strains retain an almost fixed PUR/PAR ratio. Hence, PE-rich strains of *Synechococcus* sp. better absorb available radiation under low cumulative diel photon doses during their exponential phase of growth than do PC-rich strains due to this greater plasticity of photosynthetic pigments and the placement of the absorption peaks, located around 570 nm. What is more, during the exponential phase of growth, the PE-rich strains show a much higher σPSIIʹ under low cumulative diel photon dose, and their σPSIIʹ remains higher than the PC-rich strains, even as cumulative diel photon dose increases. Hence, PE-rich strains exhibit higher light harvesting efficiency, resulting in susceptibility to higher light levels and faster light saturation compared to PC strains, particularly under the shortest (8h) and longest (24h) photoperiods.

*Synechococcus* exhibits remarkable acclimation within a strain to different environmental conditions (Śliwińska-Wilczewska et al. 2018a, 2020; Aguilera et al. 2023). Under high cumulative diel photon dose, *Synechococcus* employs photoprotective mechanisms to prevent the harmful effects of excess light energy. These include the dissipation of excess energy as heat via non-photochemical quenching (NPQ) and the regulation of phycobilisome antenna pigments, to balance light absorption and energy transfer. In contrast, under conditions of low cumulative diel PAR dose, *Synechococcus* sp. increases the expression of light-harvesting complexes to enhance light absorption (Fig. 7) and capture (Fig. 8).

Available photic regimes, combining photoperiod and peak PAR, may determine the occurrence of PC-rich and PE-rich picocyanobacterial phenotypes. Nitrogen (N) is an essential element for cyanobacteria, while the N costs to produce photosynthetic pigments varies. The molecular weight of the two phycoerythrin (PE; phycoerythrobilin) subunits is about 20,000 and 18,300 g mol−1. In turn, the molecular weight of the two phycocyanin (PC; phycocyanobilin) subunits is about 17,600 and 16,300 g mol−1 (Bennett and Bogorad 1971). The molecular weight of allophycocyanin (APC) is lower, about 16,000 g mol−1 (Bennett and Bogorad 1971) and cell-specific content of this pigment is usually very low in both phenotypes (Śliwińska-Wilczewska et al. 2020). It follows that metabolic cost of producing PE is higher than that of PC, however, it is beneficial for PE-rich picocyanobacteria, as they can capture light better than PC-rich phenotypes (Fig. 7; Fig. 8. Our results confirm that PE-rich strains are stronger light-harvesting competitors, while the PC-rich strains may have lower N-quotients for their light capture system.

## Photic regimes - implications for cumulative diel PSII electron flux

Algal dynamics respond rapidly to changes in environmental conditions (Connor 2018). We used Fast Repetition Rate fluorometry (FRRf; Fig. 3) (Kolber et al. 1998) to support an index of PSII electron transport rate per unit volume (*JV*PSII) (Suggett et al. 2003; Oxborough et al. 2012; Tortell et al. 2021). We calibrated the *JV*PSII estimator to absolute rates of electron transport measured through oxygen evolution. Using this *JV*PSII estimator we show that growth of PC-rich and PE-rich picocyanobacteria are well predicted by cumulative diel PSII electron fluxes, across different photic regimes. The growth rate, µ, of PC-rich and PE-rich picocyanobacteria shows fairly consistent saturating responses to increasing cumulative diel PSII electron flux (*JV*PSII; µmol e− µmol Chl *a*−1 d−1; Fig. 9). As previously found for diatoms (Li et al. 2017) cumulative diel reductant generation was indeed a better predictor of µ than was cumulative diel PUR, although photoperiod and peak PAR retain secondary influences on achieved growth responses of the picocyanobacteria under some conditions.

# Conclusions

We found that picocyanobacteria show different growth responses to photoperiod and light level, even under combinations giving equivalent cumulative diel PUR. Both PE-rich and PC-rich strains of *Synechococcus* sp., grew fastest under moderate light and a 24 h photoperiod. Consequently, *Synechococcus* sp. has the potential to emerge as phytoplankton components during the Arctic or Antarctic summer under future, warmed, polar regions. In optimal conditions (24 h of photoperiod and a peak PAR of 180 µmol photons m−2s−1), one of the PE-rich *Synechococcus* sp., reached the highest chlorophyll-specific exponential growth rate of 4.5 d−1 (3.7 h doubling time), a record for a cyanobacteria. PE-rich strains in the exponential phase of growth also demonstrated high ability to modulate their PUR/PAR ratio by better adjusting the pigment composition. The metabolic cost of producing PE is higher than that of PC, however, it gives them an advantage in the competition for light. We used a calibrated, fluorescence based *JV*PSII estimator, and determined that growth yields of PC-rich and PE-rich picocyanobacteria are well predicted by cumulative diel PSII electron fluxes, across different photic regimes. Differences in growth and light capture between PC-rich and PE-rich *Synechococcus* sp. could influence patterns of picocyanobacterial biomass and the composition of communities over space and time, in existing and potential aquatic environments, relating to varying metabolic costs between different physiological strategies for growth. PE-rich phenotypes of picocyanobacteria currently predominate in abundance and genetic diversity in the Baltic Sea (Aguilera et al. 2023). This dominance may be the result of eutrophication in the Baltic Sea, providing higher nitrogen for phycobiliprotein synthesis, and leading to lower light even in near-surface waters. Our results suggest possible the expansion of the range of picocyanobacteria to new photic regimes in the near future and indicate that PE-rich *Synechococcus* sp. may turn out to be the dominant component of picophytoplankton in nutrient-rich environments, allowing for high rates of phycobiliprotein synthesis.

**Additional Supporting Information may be found in the online version of this article.**

**Authors Contribution Statement:** S.S-W. designed the study with input from D.A.C. M.K. estimated the transition point between exponential and pre-stationary phase of growth. M.S. ensured the proper operation of the photobioreactors. A.A. conducted genetic analysis. N.M.O. solved technical problems related to computer operation and software. S.S-W., M.S., N.M.O., D.A.C. contributed to R coding and data analysis. S.S-W. conducted the experiments, created plots and wrote the manuscript, with support from D.A.C. All authors contributed to the discussion of the results, supported manuscript preparation, and approved the final submitted manuscript.

# Data availability statement

Data supporting this study is available on: <https://github.com/FundyPhytoPhys/BalticPhotoperiod> (public GitHub Repository) and <https://docs.google.com/spreadsheets/d/1ZXpwR7Gfto-uRzVdXzMpQF4frbrvMLH_IyLqonFZRSw/edit#gid=0> (URL for MetaDataCatalog).

Code to perform data processing and analyses is available at <https://github.com/FundyPhytoPhys/BalticPhotoperiod>.

16S rRNA sequences used in this study are available in GenBank under the accession numbers PP034393, PP034394, PP034396 and PP034403.

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## Conflict of Interest

None declared.

# References

Aguilera, A., J. Alegria Zufia, L. Bas Conn, and others. 2023. Ecophysiological analysis reveals distinct environmental preferences in closely related Baltic Sea picocyanobacteria. Environmental Microbiology **25**: 1674–1695. doi:[10.1111/1462-2920.16384](https://doi.org/10.1111/1462-2920.16384)

Arrigo, K. R. 2014. Sea ice ecosystems. Annual Review of Marine Science **6**: 439–467. doi:[10.1146/annurev-marine-010213-135103](https://doi.org/10.1146/annurev-marine-010213-135103)

Beale, S. I. 1994. [Biosynthesis of cyanobacterial tetrapyrrole pigments: Hemes, chlorophylls, and phycobilins](https://doi.org/10.1007/978-94-011-0227-8_17), p. 519–558. *In* D.A. Bryant [ed.], The Molecular Biology of Cyanobacteria. Springer Netherlands.

Behrenfeld, M. J., R. T. O’Malley, D. A. Siegel, and others. 2006. Climate-driven trends in contemporary ocean productivity. Nature **444**: 752–755. doi:[10.1038/nature05317](https://doi.org/10.1038/nature05317)

Bennett, A., and L. Bogorad. 1971. Properties of subunits and aggregates of blue-green algal biliproteins. Biochemistry **10**: 3625–3634. doi:[10.1021/bi00795a022](https://doi.org/10.1021/bi00795a022)

Bennett, A., and L. Bogorad. 1973. Complementary Chromatic Adaptation in a filamentous blue-green alga. Journal of Cell Biology **58**: 419–435. doi:[10.1083/jcb.58.2.419](https://doi.org/10.1083/jcb.58.2.419)

Boatman, T. G., R. J. Geider, and K. Oxborough. 2019. Improving the accuracy of Single Turnover Active Fluorometry (STAF) for the estimation of Phytoplankton Primary Productivity (PhytoPP). Frontiers in Marine Science **6**. doi:[10.3389/fmars.2019.00319](https://doi.org/10.3389/fmars.2019.00319)

Brand, L. E., and R. R. L. Guillard. 1981. The effects of continuous light and light intensity on the reproduction rates of twenty-two species of marine phytoplankton. Journal of Experimental Marine Biology and Ecology **50**: 119–132. doi:[10.1016/0022-0981(81)90045-9](https://doi.org/10.1016/0022-0981(81)90045-9)

Chakdar, H., and S. Pabbi. 2016. [Cyanobacterial phycobilins: Production, purification, and regulation](https://doi.org/10.1007/978-81-322-2610-9_4), p. 45–69. *In* P. Shukla [ed.], Frontier Discoveries and Innovations in Interdisciplinary Microbiology. Springer India.

Christaki, U., S. Jacquet, J. R. Dolan, D. Vaulot, and F. Rassoulzadegan. 1999. Growth and grazing on *Prochlorococcus* and *Synechococcus* by two marine ciliates. Limnology and Oceanography **44**: 52–61. doi:[10.4319/lo.1999.44.1.0052](https://doi.org/10.4319/lo.1999.44.1.0052)

Connor, D. 2018. Investigating the use of fast repetition rate fluorometry in understanding algal physiology in optically complex oceans.

Elzhov, T. V., K. M. Mullen, A.-N. Spiess, and B. Bolker. 2023. Minpack.lm: R Interface to the Levenberg-Marquardt Nonlinear Least-Squares Algorithm Found in MINPACK, Plus Support for Bounds.

Erga, S. R., and B. R. Heimdal. 1984. Ecological studies on the phytoplankton of Korsfjorden, western Norway. The dynamics of a spring bloom seen in relation to hydrographical conditions and light regime. Journal of Plankton Research **6**: 67–90. doi:[10.1093/plankt/6.1.67](https://doi.org/10.1093/plankt/6.1.67)

Falkowski, P., and Z. Kolber. 1993. Estimation of phytoplankton photosynthesis by active fluorescence. ICES Marine Science Symposium **197**: 92–103.

Field, C. B., M. J. Behrenfeld, J. T. Randerson, and P. Falkowski. 1998. Primary production of the biosphere: Integrating terrestrial and oceanic components. Science **281**: 237–240. doi:[10.1126/science.281.5374.237](https://doi.org/10.1126/science.281.5374.237)

Flombaum, P., J. L. Gallegos, R. A. Gordillo, and others. 2013. Present and future global distributions of the marine Cyanobacteria *Prochlorococcus* and *Synechococcus*. Proceedings of the National Academy of Sciences **110**: 9824–9829. doi:[10.1073/pnas.1307701110](https://doi.org/10.1073/pnas.1307701110)

Gradinger, R., and J. Lenz. 1989. Picocyanobacteria in the high Arctic. Marine Ecology Progress Series **52**: 99–101. doi:[10.3354/meps052099](https://doi.org/10.3354/meps052099)

Guillard, R. R. L. 1975. [Culture of phytoplankton for feeding marine invertebrates](https://doi.org/10.1007/978-1-4615-8714-9_3), p. 29–60. *In* W.L. Smith and M.H. Chanley [eds.], Culture of Marine Invertebrate Animals: Proceedings — 1st Conference on Culture of Marine Invertebrate Animals Greenport. Springer US.

Handel, A. 2020. Andreas Handel - Custom Word formatting using R Markdown.

Harrison, W. G., and T. Platt. 1986. Photosynthesis-irradiance relationships in polar and temperate phytoplankton populations. Polar biology **5**: 153–164.

Haverkamp, T. H. A., D. Schouten, M. Doeleman, U. Wollenzien, J. Huisman, and L. J. Stal. 2009. Colorful microdiversity of *Synechococcus* strains (picocyanobacteria) isolated from the Baltic Sea. The ISME Journal **3**: 397–408. doi:[10.1038/ismej.2008.118](https://doi.org/10.1038/ismej.2008.118)

Hirata, T., N. J. Hardman-Mountford, R. J. W. Brewin, and others. 2011. Synoptic relationships between surface Chlorophyll-*a* and diagnostic pigments specific to phytoplankton functional types. Biogeosciences **8**: 311–327. doi:[10.5194/bg-8-311-2011](https://doi.org/10.5194/bg-8-311-2011)

Hoang, D. T., O. Chernomor, A. von Haeseler, B. Q. Minh, and L. S. Vinh. 2018. UFBoot2: Improving the Ultrafast Bootstrap Approximation. Molecular Biology and Evolution **35**: 518–522. doi:[10.1093/molbev/msx281](https://doi.org/10.1093/molbev/msx281)

Holtrop, T., J. Huisman, M. Stomp, and others. 2021. Vibrational modes of water predict spectral niches for photosynthesis in lakes and oceans. Nature Ecology & Evolution **5**: 55–66. doi:[10.1038/s41559-020-01330-x](https://doi.org/10.1038/s41559-020-01330-x)

Huisman, J., M. Arrayás, U. Ebert, and B. Sommeijer. 2002. How do sinking phytoplankton species manage to persist? The American Naturalist **159**: 245–254. doi:[10.1086/338511](https://doi.org/10.1086/338511)

Jacob-Lopes, E., C. H. G. Scoparo, L. M. C. F. Lacerda, and T. T. Franco. 2009. Effect of light cycles (night/day) on CO2 fixation and biomass production by microalgae in photobioreactors. Chemical Engineering and Processing: Process Intensification **48**: 306–310. doi:[10.1016/j.cep.2008.04.007](https://doi.org/10.1016/j.cep.2008.04.007)

Jávorfi, T., J. Erostyák, J. Gál, A. Buzády, L. Menczel, G. Garab, and K. Razi Naqvi. 2006. Quantitative spectrophotometry using integrating cavities. Journal of Photochemistry and Photobiology B: Biology **82**: 127–131. doi:[10.1016/j.jphotobiol.2005.10.002](https://doi.org/10.1016/j.jphotobiol.2005.10.002)

Kalyaanamoorthy, S., B. Q. Minh, T. K. F. Wong, A. von Haeseler, and L. S. Jermiin. 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods **14**: 587–589. doi:[10.1038/nmeth.4285](https://doi.org/10.1038/nmeth.4285)

Katoh, K., J. Rozewicki, and K. D. Yamada. 2019. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics **20**: 1160–1166. doi:[10.1093/bib/bbx108](https://doi.org/10.1093/bib/bbx108)

Klepacz-Smółka, A., D. Pietrzyk, R. Szeląg, P. Głuszcz, M. Daroch, J. Tang, and S. Ledakowicz. 2020. Effect of light colour and photoperiod on biomass growth and phycocyanin production by *Synechococcus* PCC 6715. Bioresource Technology **313**: 123700. doi:[10.1016/j.biortech.2020.123700](https://doi.org/10.1016/j.biortech.2020.123700)

Kolber, Z. S., O. Prášil, and P. G. Falkowski. 1998. Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: Defining methodology and experimental protocols. Biochimica et Biophysica Acta (BBA) - Bioenergetics **1367**: 88–106. doi:[10.1016/S0005-2728(98)00135-2](https://doi.org/10.1016/S0005-2728(98)00135-2)

Krishnan, A., M. Likhogrud, M. Cano, and others. 2021. *Picochlorum Celeri* as a model system for robust outdoor algal growth in seawater. Scientific Reports **11**: 11649. doi:[10.1038/s41598-021-91106-5](https://doi.org/10.1038/s41598-021-91106-5)

Lane, D. J. 1991. 16S/23S rRNA sequencing. Nucleic acid techniques in bacterial systematics 115–175.

LaRoche, J., and B. M. Robicheau. 2022. [The Pelagic Light-Dependent Microbiome](https://doi.org/10.1007/978-3-030-90383-1_9), p. 395–423. *In* L.J. Stal and M.S. Cretoiu [eds.], The Marine Microbiome. Springer International Publishing.

Li, G., D. Talmy, and D. A. Campbell. 2017. Diatom growth responses to photoperiod and light are predictable from diel reductant generation. Journal of Phycology **53**: 95–107. doi:[10.1111/jpy.12483](https://doi.org/10.1111/jpy.12483)

Li, Q., L. Legendre, and N. Jiao. 2015. Phytoplankton responses to nitrogen and iron limitation in the tropical and subtropical Pacific Ocean. Journal of Plankton Research **37**: 306–319. doi:[10.1093/plankt/fbv008](https://doi.org/10.1093/plankt/fbv008)

Li, W. K. W. 1995. [Composition of ultraphytoplankton in the central North Atlantic](https://www.jstor.org/stable/24852252). Marine Ecology Progress Series **122**: 1–8.

Ligges, U., T. Short, P. Kienzle, and others. 2024. Signal: Signal Processing.

Litchman, E., C. A. Klausmeier, and K. Yoshiyama. 2009. Contrasting size evolution in marine and freshwater diatoms. Proceedings of the National Academy of Sciences **106**: 2665–2670. doi:[10.1073/pnas.0810891106](https://doi.org/10.1073/pnas.0810891106)

Longobardi, L., L. Dubroca, F. Margiotta, D. Sarno, and A. Zingone. 2022. Photoperiod-driven rhythms reveal multi-decadal stability of phytoplankton communities in a highly fluctuating coastal environment. Scientific Reports **12**: 3908. doi:[10.1038/s41598-022-07009-6](https://doi.org/10.1038/s41598-022-07009-6)

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

Minh, B. Q., H. A. Schmidt, O. Chernomor, D. Schrempf, M. D. Woodhams, A. von Haeseler, and R. Lanfear. 2020. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Molecular Biology and Evolution **37**: 1530–1534. doi:[10.1093/molbev/msaa015](https://doi.org/10.1093/molbev/msaa015)

Moejes, F. W., A. Matuszyńska, K. Adhikari, and others. 2017. A systems-wide understanding of photosynthetic acclimation in algae and higher plants. Journal of Experimental Botany **68**: 2667–2681. doi:[10.1093/jxb/erx137](https://doi.org/10.1093/jxb/erx137)

Morel, A. 1978. Available, usable, and stored radiant energy in relation to marine photosynthesis. Deep Sea Research **25**: 673–688. doi:[10.1016/0146-6291(78)90623-9](https://doi.org/10.1016/0146-6291(78)90623-9)

Morel, A. 1988. Optical modeling of the upper ocean in relation to its biogenous matter content (case I waters). Journal of Geophysical Research: Oceans **93**: 10749–10768. doi:[10.1029/JC093iC09p10749](https://doi.org/10.1029/JC093iC09p10749)

Oxborough, K., and N. R. Baker. 1997. Resolving chlorophyll *a* fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components – calculation of qP and Fv-/Fm-; without measuring Fo-; Photosynthesis Research **54**: 135–142. doi:[10.1023/A:1005936823310](https://doi.org/10.1023/A:1005936823310)

Oxborough, K., C. M. Moore, D. J. Suggett, T. Lawson, H. G. Chan, and R. J. Geider. 2012. Direct estimation of functional PSII reaction center concentration and PSII electron flux on a volume basis: A new approach to the analysis of Fast Repetition Rate fluorometry (FRRf) data. Limnology and Oceanography: Methods **10**: 142–154. doi:[10.4319/lom.2012.10.142](https://doi.org/10.4319/lom.2012.10.142)

Pedersen, T. L. 2024. Patchwork: The Composer of Plots.

Pick, F. R. 1991. The abundance and composition of freshwater picocyanobacteria in relation to light penetration. Limnology and Oceanography **36**: 1457–1462. doi:[10.4319/lo.1991.36.7.1457](https://doi.org/10.4319/lo.1991.36.7.1457)

Posit team. 2022. [RStudio: Integrated development environment for r](http://www.posit.co/), Posit Software, PBC.

R Core Team. 2023. [R: A language and environment for statistical computing](https://www.R-project.org/), R Foundation for Statistical Computing.

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

Ryan, J. A., J. M. Ulrich, R. Bennett, and C. Joy. 2024. Xts: eXtensible Time Series.

Sakamoto, T., and D. A. Bryant. 1999. Nitrate transport and not photoinhibition limits growth of the freshwater cyanobacterium *Synechococcus* species PCC 6301 at low temperature. Plant Physiology **119**: 785–794. doi:[10.1104/pp.119.2.785](https://doi.org/10.1104/pp.119.2.785)

Schuurmans, R. M., J. C. P. Matthijs, and K. J. Hellingwerf. 2017. Transition from exponential to linear photoautotrophic growth changes the physiology of *Synechocystis* sp. PCC 6803. Photosynthesis Research **132**: 69–82. doi:[10.1007/s11120-016-0329-8](https://doi.org/10.1007/s11120-016-0329-8)

Serway, R. A., C. J. Moses, and C. A. Moyer. 2004. Modern Physics, Cengage Learning.

Six, C., Z. V. Finkel, A. J. Irwin, and D. A. Campbell. 2007. Light variability illuminates niche-partitioning among marine picocyanobacteria. PLOS ONE **2**: e1341. doi:[10.1371/journal.pone.0001341](https://doi.org/10.1371/journal.pone.0001341)

Six, C., M. Ratin, D. Marie, and E. Corre. 2021. Marine *Synechococcus* picocyanobacteria: Light utilization across latitudes. Proceedings of the National Academy of Sciences **118**: e2111300118. doi:[10.1073/pnas.2111300118](https://doi.org/10.1073/pnas.2111300118)

Śliwińska-Wilczewska, S., A. Cieszyńska, J. Maculewicz, and A. Latała. 2018a. Ecophysiological characteristics of red, green, and brown strains of the Baltic picocyanobacterium *Synechococcus* sp. – a laboratory study. Biogeosciences **15**: 6257–6276. doi:[10.5194/bg-15-6257-2018](https://doi.org/10.5194/bg-15-6257-2018)

Śliwińska-Wilczewska, S., Z. Konarzewska, K. Wiśniewska, and M. Konik. 2020. Photosynthetic pigments changes of three phenotypes of picocyanobacteria *Synechococcus* sp. Under different light and temperature conditions. Cells **9**: 2030. doi:[10.3390/cells9092030](https://doi.org/10.3390/cells9092030)

Śliwińska-Wilczewska, S., J. Maculewicz, A. Barreiro Felpeto, and A. Latała. 2018b. Allelopathic and bloom-forming picocyanobacteria in a changing world. Toxins **10**: 48. doi:[10.3390/toxins10010048](https://doi.org/10.3390/toxins10010048)

Stadnichuk, I. N., P. M. Krasilnikov, and D. V. Zlenko. 2015. Cyanobacterial phycobilisomes and phycobiliproteins. Microbiology **84**: 101–111. doi:[10.1134/S0026261715020150](https://doi.org/10.1134/S0026261715020150)

Strickland, J. D., and T. R. Parsons. 1972. Practical Hand Book of Seawater Analysis. Fisheries Research Board of Canada **167 (2nd edition)**: 1–311. doi:<DOI: http://dx.doi.org/10.25607/OBP-1791>

Suggett, D. J., K. Oxborough, N. R. Baker, H. L. MacIntyre, T. M. Kana, and R. J. Geider. 2003. Fast repetition rate and pulse amplitude modulation chlorophyll *a* fluorescence measurements for assessment of photosynthetic electron transport in marine phytoplankton. European Journal of Phycology **38**: 371–384. doi:[10.1080/09670260310001612655](https://doi.org/10.1080/09670260310001612655)

Theus, M. E., T. J. Layden, N. McWilliams, S. Crafton-Tempel, C. T. Kremer, and S. B. Fey. 2022. Photoperiod influences the shape and scaling of freshwater phytoplankton responses to light and temperature. Oikos **2022**: e08839. doi:[10.1111/oik.08839](https://doi.org/10.1111/oik.08839)

Torremorell, A., M. E. Llames, G. L. Pérez, R. Escaray, J. Bustingorry, and H. Zagarese. 2009. Annual patterns of phytoplankton density and primary production in a large, shallow lake: The central role of light. Freshwater Biology **54**: 437–449. doi:[10.1111/j.1365-2427.2008.02119.x](https://doi.org/10.1111/j.1365-2427.2008.02119.x)

Tortell, P., D. J. Suggett, and S. W. Group156. 2021. [A user guide for the application of Single Turnover active chlorophyll fluorescence for phytoplankton productivity measurements. Version 1.](https://doi.org/10.25607/OBP-1084) Report Scientific Committee on Oceanic Research (SCOR) Working Group 156.

van Alphen, P., H. Abedini Najafabadi, F. Branco dos Santos, and K. J. Hellingwerf. 2018. Increasing the photoautotrophic growth rate of *Synechocystis* sp. PCC 6803 by identifying the limitations of its cultivation. Biotechnology Journal **13**: 1700764. doi:[10.1002/biot.201700764](https://doi.org/10.1002/biot.201700764)

Velichko, N., S. Smirnova, S. Averina, and A. Pinevich. 2021. A survey of Antarctic cyanobacteria. Hydrobiologia **848**: 2627–2653.

Vincent, W. F., J. P. Bowman, L. M. Rankin, and T. A. McMeekin. 2000. Phylogenetic diversity of picocyanobacteria in Arctic and Antarctic ecosystems. Microbial biosystems: new frontiers. Proceedings of the 8th International Symposium on Microbial Ecology 317–322.

Wickham, H. 2016. [Data Analysis](https://doi.org/10.1007/978-3-319-24277-4_9), p. 189–201. *In* H. Wickham [ed.], Ggplot2: Elegant Graphics for Data Analysis. Springer International Publishing.

Wood, S. N. 2017. [Generalized Additive Models: An Introduction with R, Second Edition](https://doi.org/10.1201/9781315370279), 2nd ed. Chapman and Hall/CRC.

Xi, H., S. N. Losa, A. Mangin, and others. 2020. Global retrieval of phytoplankton functional types based on empirical orthogonal functions using CMEMS GlobColour merged products and further extension to OLCI data. Remote Sensing of Environment **240**: 111704. doi:[10.1016/j.rse.2020.111704](https://doi.org/10.1016/j.rse.2020.111704)

Xu, K., J. L. Grant-Burt, N. Donaher, and D. A. Campbell. 2017. Connectivity among Photosystem II centers in phytoplankters: Patterns and responses. Biochimica et Biophysica Acta (BBA) - Bioenergetics **1858**: 459–474. doi:[10.1016/j.bbabio.2017.03.003](https://doi.org/10.1016/j.bbabio.2017.03.003)

Xu, K., J. Lavaud, R. Perkins, E. Austen, M. Bonnanfant, and D. A. Campbell. 2018. Phytoplankton PSII and excitation dissipation; implications for estimates of primary productivity. Frontiers in Marine Science **5**. doi:[10.3389/fmars.2018.00281](https://doi.org/10.3389/fmars.2018.00281)