Coastal picocyanobacteria can exploit low oxygen habitats

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# Abstract

*Synechococcus* sp., one of the most abundant primary producers in marine ecosystems, exhibit remarkable adaptations to thrive in low oxygen environments, such as Oxygen Minimum Zones (OMZs) and the wide range of spectral wavebands, prevalent at depths in the world’s ocean. We found that PhycoCyanin(PC)-rich and PhycoErythrin(PE)-rich *Synechococcus* sp. are able to grow under 2.5 µM oxygen concentration [O2], across the range of tested spectral wavebands from 405 – 730 nm. The PC-rich strain showed generally similar chlorophyll-specific growth rates (µ; d−1) under tested conditions, whereas PE-rich cultures achieved faster growth rates under 2.5 µM [O2] than under 250 µM [O2]. As expected, PC-rich strain showed a peak in growth rate under red light (620 or 660 nm). However, under 2.5 µM [O2] the PE-rich strain showed similar and high growth rates under 530 nm – 660 nm. Here, we also showed that the pigment content varies depending on the [O2] and growth wavebands. PC-rich *Synechococcus* sp. had higher phycobiliproteins content under 250 µM [O2], while for PE-rich strain, the highest phycobiliproteins content was noted under 620 nm and 2.5 µM [O2]. For PC-rich and PE-rich *Synechococcus* sp., µ showed also positive linear responses to both Phycobiliproteins:Chlorophyll *a* ratio, and cumulative diel PSII electron flux (*JV*PSII), although the relations vary across strain and [O2]. Under orange excitation at Ex590nm, σPSII was higher at 250 µM [O2] compared to 2.5 µM [O2] for the PC-rich strain, on the contrary, what was shown by PE-rich culture. τPSII, was generally faster and qP was generally higher for both PC-rich and PE-rich strains under 250 µM [O2], since cyanobacteria show strong capacity for electron flow away from PSII to O2, particularly under excess excitation. PE-rich *Synechococcus* sp. are typically found at greater depths, and lower light, than are PC-rich strains but we suggest that the PE-rich strains are actually limited to lower light by an interaction between light and full air-saturated [O2]. In lower oxygen waters PE-rich strains are likely able to exploit higher light niches.

**Key words:** OMZs, oxygen concentration, spectral wavebands, *Synechococcus*, PC-rich strain, PE-rich strain

# Introduction

Oxygen is essential for the survival of organisms and regulates the global cycling of nutrients and carbon. However, oxygen levels in the open ocean and coastal waters have declined over the past half-century due to human activities that increase global temperatures and nutrient release (Breitburg et al. 2018). Since the mid-20th century, ocean deoxygenation has become a critical change in marine ecosystems, affecting productivity, biodiversity and biogeochemical cycles. This ocean deoxygenation ranks among the most important changes occurring in marine ecosystems (Keeling et al. 2010). Over the past 50 years, the open ocean has lost about 2% of oxygen, and models predict a further decline of several percent by the end of the century, which could cause significant biogeochemical and ecological impacts. Oxygen minimum zones (OMZs) in the open ocean have expanded to an area equivalent to the European Union, and the volume of oxygen-free water has quadrupled (Breitburg et al. 2018). Due to this fact, it has become necessary to research and forecast which species will be able to survive and dominate under the influence of the predicted and ongoing changes in the ecosystem.

Picocyanobacteria numerically dominate vast tracts of the world’s oceans, particularly in oligotrophic regions exhibit remarkable adaptations to thrive in low oxygen environments, such as OMZs, prevalent at depths in the ocean (Wong et al. 2023). These zones are characterized by exceptionally low dissolved oxygen concentrations, posing challenges for aerobic organisms. However, picocyanobacteria species have evolved diverse strategies to cope with these conditions. Picocyanobacteria, can perform photosynthesis under extremely low light and oxygen levels, utilizing specialized pigments and photosystems to capture and utilize light energy efficiently (Murphy et al. 2017; Luimstra et al. 2018). Furthermore, recent studies have highlighted the role of specific genetic adaptations in cyanobacteria populations inhabiting OMZs, enabling them to tolerate and even thrive in oxygen-depleted environments. These adaptations encompass genetic modifications related to energy metabolism, antioxidant defense mechanisms, and cellular structures optimized for oxygen scavenging and storage (Ulloa et al. 2012). Overall, the occurrence and adaptation of picocyanobacteria to low oxygen environments are critical components of marine ecosystems, shaping their productivity and biodiversity in OMZ regions.

Another important factor that should be considered when predicting the occurrence of dominant picocyanobacteria in the water column during changing environmental conditions is their adaptation to various spectral wavebands, ranging from short-wavelength blue light, through green and yellow light to long-wavelength red light. Despite competition theory often overlooking the light spectrum as a significant axis of niche differentiation, plankton ecologists have long acknowledged that the diverse array of photosynthetic pigments allows cyanobacteria species to exploit different spectral wavebands (Falkowski et al. 2004; Stomp et al. 2007). Picocyanobacteria from the genus *Synechococcus* includes clusters rich in phycoerythrin (PE), which imparts a range of orange, reddish, pink, and purple colors, as well as phenotypes rich in phycocyanin (PC), which color the organism in various shades of blue-green. Competition experiments have demonstrated that PE-rich and PC-rich strains can coexist in white light through spectral niche differentiation (Stomp et al. 2004). This differentiation in spectral niches can also elucidate the distribution patterns of reddish and blue-green strains in natural waters. PE-rich strains dominate in clear waters due to their relatively high content of the chromophore phycourobilin (PUB) in the clearest ocean waters where blue light predominates, while their chromophore composition shifts towards phycoerythrobilin (PEB) in more mesotrophic marine waters characterized by blue-green light environments. Conversely, PC-rich strains prevail in turbid waters where orange and red light dominate. The widespread coexistence of PE-rich and PC-rich picocyanobacteria is observed in waters of intermediate turbidity, such as mesotrophic lakes and coastal seas (Haverkamp et al. 2009).

Picocyanobacteria, are the most abundant primary producers in marine ecosystems (Flombaum et al. 2013). The ecological success of picoplanktonic *Synechococcus* sp. to occur throughout the water column suggests they possess sophisticated strategies to respond to variations in their environment (Scanlan 2012), especially to different oxygen concentrations and colors of light. Thus, the aim of this work was to demonstrate the ecophysiological response of PC-rich and PE-rich *Synechococcus* sp. cultures to different oxygen concentrations (250 µM or 2.5 µM [O2]) and spectral wavebands from 405 – 730 nm. In this work, we empirically answer the question posed by Wong et al. (2023) regarding the sensitivity of modern picocyanobacteria to low levels of O2 and the wide range of the light spectrum found at greater depths, and which species will dominate in these waters in the near future.

# Materials and methods

## Culture condition and experimental setup

Xenic cultures of PC-rich (CCBA\_077) and PE-rich (CCBA\_127) *Synechococcus* sp. were obtained from the Culture Collection of Baltic Algae (<https://ccba.ug.edu.pl/pages/en/home.php>) (Latala et al. 2006). *Synechococcus* sp. strains were cultured in Tissue Culture Flasks (VWR International, Cat. No. 10062-872, PA, USA) and transferred biweekly to fresh f/2 media (Guillard 1975) prepared at a salinity of 8 PSU, reflective of their natural brackish habitat. Pre-cultures were maintained in incubators with full air saturated dissolved oxygen concentration [O2] of 250 µM, 22℃, with a light/dark cycle of 12 h and Photosynthetically Active Radiation (PAR) of 10 µmol photons m−2s−1 from Philips Cool White F14T5/841 Alto, 14 watts, fluorescent bulbs.

Controlled growth experiments were performed using MCMIX-OD PSI Multicultivators (Photon Systems Instruments, Drásov, Czech Republic) set to 22℃. Each of 8 round bottom cylindrical glass tubes contained 75 mL of f/2 medium and 5 mL of growing pre-culture. These parameters allowed for exponential growth of the cultures from the beginning of the experiment, with little lag phase. Inoculation of culture tubes took place in the afternoon, with a period of low light and then 12 h darkness before a sinusoidal 12 h photoperiod cycle commenced at 07:00 the following morning, with peak PAR of 180 µmol photons m−2s−1 reached at 13:00 each day.

Each tube was maintained under an individual combination of one of 7 spectral wavebands (centred at 405, 450, 470, 530, 620, 660, or 730 nm); at 250 µM or 2.5 µM [O2]. 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. We used aeration with a total gas flow rate of around ~ 140 mL min−1 tube−1 through a 0.2µm sterile microfilter provided via a G400 gas mixing system (Qubit Systems Inc., Kingston, Ontario, Canada). ~ 250 µM [O2] was achieved by sparging with lab air (78% N2, 21% O2, 1% Ar and 0.05% CO2). ~ 2.5 µM [O2] was achieved by sparging with a gas mixture containing 99.95% N2 and 0.05% CO2. [O2] *in situ* was verified using oxygen optodes (PyroScience, Germany) inserted into tubes for real-time measurements (data not presented), with software correction to account for the salinity of the media (8 PSU). The pH of tested cultures remained about 8, with limited fluctuation during the growth experiment (data not presented).

## Chlorophyll-specific growth rates

Picocyanobacterial growth was monitored every 5 minutes by automatically recording OD680, OD720, and ΔOD (ΔOD = OD680 – OD720) for at least 5 days, independently for each culture tube. The chlorophyll-specific 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 (d) for each combination of strain, spectral waveband, and [O2]. Growth curves, tracked as OD680, OD720, ΔOD and logistic fits of ΔOD vs. elapsed time are shown in Fig. S1 in Supplementary materials. Cell-specific growth rates (µ) were also determined using OD720 (Fig. S2).

## Picocyanobacteria cell counts

Picocyanobacterial cells mL−1 were estimated using linear regression models of OD at 680 nm or 720 nm vs. calibration counts of cell suspension densities (cell mL−1) (Table S1). The OD of cultures was measured using MCMIX-OD PSI Multicultivators (Photon Systems Instruments, Drásov, Czech Republic) and cell suspension density measures were conducted 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 microscopy measures. Fixed samples of culture (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 swinging 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 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 mL−1 was calculated based on the dilution factor and selected count area from each well (Wlodkowic et al. 2022).

## Pigment content and pigment ratio

Whole-cell absorbance spectra of picocyanobacteria cells were collected using an integrating cavity spectrophotometer (CLARiTY 17 UV/Vis/NIR, On-Line Instrument Systems, Inc., Bogart, GA, USA) according to the method proposed by Blake and Griff (2012). The sample and reference observation cavities of the spectrophotometer were filled with 8 mL of f/2 medium at salinity 8 PSU. After establishing a baseline absorbance spectra from 375 to 710 nm, 4 mL culture medium was replaced with 4 mL of culture in the sample cavity. Pathlength corrected absorbance per cm was calculated using Jávorfi coefficients (Jávorfi et al. 2006). We then conducted estimations of pigment content (µg mL−1) including Chlorophyll *a* (Chl *a*), Carotenoids (Car), Phycoerythrin (PE), Phycocyanin (PC), and Allophycocyanin (APC) from the PC-rich and PE-rich *Synechococcus* sp. cultures. These estimations were based on established linear correlations between pigment content, determined through extraction methods (Strickland and Parsons 1972; Bennett and Bogorad 1973), and absorbance values of individual pigment peaks (Car; 480 nm, PE; 565 nm, PC; 620 nm, APC; 650 nm, and Chl *a*; 665 nm) obtained from whole-cell absorbance spectra (Table S2). We also summed PE, PC, and APC protein to total Phycobiliproteins content. We then calculated the Car to Chl *a* ratio, and the ratio of the sum of Phycobiliproteins to Chl *a* (µg:µg) for each strain (Fig. S3).

## PSII effective absorption cross section of PSII, turnover time of PSII photochemistry, and photochemical quenching coefficient

We harvested 4 mL of picocyanobacteria cultures repeatedly across the growth trajectories for photophysiological characterizations. For the low oxygen cultures, to ensure photophysiological measurements were taken at low O2 of ~ 2.5 µM, we bubbled gently with N2 from a gas cylinder during measurements. [O2] was verified in culture samples for photophysiological measurements using oxygen optodes (PyroScience, Germany) inserted (data not presented).

We used Fast Repetition & Relaxation chlorophyll fluorescence (FRRf) (Kolber et al. 1998) (Solisense, USA), with a lab built temperature control jacket (22℃), to apply a series of 100 excitation flashlets of 1.6 µs to drive saturation of PSII variable fluorescence, followed immediately by logarithmically spaced flashlets to track relaxation of variable fluorescence. Induction/relaxation trajectories were fit using the onboard Solisense LIFT software (Falkowski and Kolber 1993; Kolber et al. 1998).

We used a double tap protocol (Xu et al. 2017), where FRRf induction/relaxation trajectories were collected during a rapid actinic light curve sequence increasing in steps of 10 s at 0, 20, 40, 80, 160, and 320 µmol photons m−2s−1 PAR. We applied 1 s darkness between the sequential 10 s steps of the light response curves, to allow re-opening of PSII immediately after application of the sequential increasing light steps. Flashlets and actinic light were delivered from LED emitters centred at Ex445nm, preferentially exciting chlorophyll; Ex470nm,, preferentially exciting phycourobilin (PUB); Ex535nm, preferentially exciting phycoerythrin (PE); or Ex590nm, preferentially exciting phycocyanin (PC). Excitation flashlets and actinic light wavebands were matched for each run. These actinic and excitation wavebands in turn approximated 4 of our 7 growth light wavebands (450, 470, 530 & 620 nm), allowing us to evaluate *in situ* photosynthetic performance for those culture conditions.

Flashlet power delivered to the samples during the 1.6 µs flashlet duration was adjusted to achieve saturation of variable fluorescence; Ex445nm at 60000 µmol photons m−2s−1 PAR; Ex470nm at 30000 µmol photons m−2s−1 PAR; Ex535nm at 25000 µmol photons m−2s−1 PAR; while for Ex590nm excitation power at 14000 µmol photons m−2s−1, calibrated using a quantum sensor (LI-250, LI-COR, Inc.) in the temperature controlled cuvette.

We estimated effective absorption cross section of PSII (σPSII; nm2 quanta−1); turnover time of PSII photochemistry (τPSII; µs); and the photochemical quenching coefficient (qP) using the FRRf induction curves, following (Xu et al. 2017).

3C3PSII (Fig. S4); 3C4PSII (Fig. S5); or qP (Fig. S6) vs. Actinic PAR (µmol photons m−2s−1) under excitation at Ex445nm, blue; Ex470nm, blue-green; Ex535nm, green; or Ex590nm, orange; for PC-rich or PE-rich cultures of *Synechococcus* sp. grown at spectral bandwidths of 450, 470, 530, or 620 nm and O2 concentrations of 250 µM or 2.5 µM are shown in Supplementary materials.

## PSII electron flux

We calculated (Eq. (1)) an uncalibrated fluorescence based estimator for volumetric electron transport, *JV*PSII, (k × e− L−1 s−1) under Ex445nm, blue; Ex470nm, blue-green; Ex535nm, green; or Ex590nm, red-orange excitation bands (Oxborough et al. 2012; Boatman et al. 2019; Tortell and Suggett 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 waveband (relative fluorescence) and σPSII is the maximum effective absorption cross section for PSII photochemistry from a given sample and excitation waveband (nm2 quanta−1).

We calibrated the *JV*PSII estimator to absolute rates of electron transport (Eq. (2)) using parallel measures of oxygen evolution (µmol O2 L−1 s−1), captured simultaneously with the FRRf measures, taken below light saturation of electron transport to limit distortion from electron fluxes back to oxygen under super-saturating light (Hughes et al. 2018), using a FireSting robust oxygen probe (PyroScience, Germany) inserted in the cuvette for select Rapid Light Curve (RLC) runs (Table S3).

PSII electron flux (*JV*PSII; µmol e− L−1 s−1) vs. Actinic PAR (µmol photons m−2s−1) estimated using FRRf induction curves with excitation at Ex445nm, blue; Ex470nm, blue-green; Ex535nm, green; or Ex590nm, orange; for PC-rich or PE-rich cultures of *Synechococcus* sp. grown at spectral bandwidths of 450, 470, 530, or 620 nm and O2 concentrations of 250 µM or 2.5 µM are also presented (Fig. S7).

Next, we converted *JV*PSII (µmol e− L−1 s−1) to *JV*PSII (µmol e− µmol Chl *a*−1 d−1) by performing Chl *a* (µg L−1) measurements using Trilogy Laboratory Fluorometer (Turner Designs, Inc., CA, USA) equipped with Chlorophyll In-Vivo Module, at the same time as the FRRf measurements.

## Statistical analysis

We used R version 4.3.0 (R Core Team 2023) running under RStudio (Posit team 2022). We performed three-way factorial ANOVA (*aov()* function; R Base package) to determine whether strain, growth waveband, and [O2] significantly influence the chlorophyll-specific growth rate (µ; d−1; Tab. S4) vs. pigment content (Tab. S5). We also performed three-way factorial ANOVA (*aov()* function) to determine whether strain, Actinic PAR, and [O2] significantly influence the responses of σPSII (Tab. S6); τPSII (Tab. S7); qP (Tab. S8); or *JV*PSII (Tab. S9) to increasing light.

We used *t*-tests of linear regressions to compare data across different strains and [O2] for a given growth waveband, for chlorophyll-specific growth rate vs. Phycobiliproteins to Chl *a* ratio (Tab. S10). We also performed *t*-tests of linear fits to compare data across different strains and [O2] in situations in which cultures were excited by, and growing in, corresponding growth wavebands of 450, 470, 530, or 620 nm, for chlorophyll-specific growth rate vs. *JV*PSII (Tab. S11). 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 ggplot2 (Wickham 2016) and patchwork (Pedersen 2024) packages. All metadata, data, and code is available on GitHub (<https://github.com/FundyPhytoPhys/BalticO2>).

# Results

## Chlorophyll-specific growth rates across [O2], spectral wavebands, and strains

We used logistic curve fits (Fig. S1) to determine chlorophyll-specific growth rates (μ; d−1) for PC-rich and PE-rich cultures of *Synechococcus* sp. grown under spectral wavebands centred at 405, 450, 470, 530, 620, 660, or 730 nm, and [O2] of 250 µM or 2.5 µM (Fig. 1). Cell-specific growth rates (µ) were also determined using OD720 (Fig. S2). Strain, growth waveband, [O2], and their interactions, significantly affected μ (Table S4). PC-rich and PE-rich *Synechococcus* sp. grow under 2.5 µM [O2], across the range of tested spectral wavebands from 405 – 730 nm. In contrast, under 250 µM [O2], the PC-rich strain failed to grow under 405 nm, while the PE-rich strain failed to grow under 405, 450, and 730 nm. The PC-rich strain showed generally similar growth rates under 2.5 and 250 µM [O2], across tested spectral wavebands (nm). In contrast the PE-rich strain achieved faster growth rates under 2.5 µM [O2] than under 250 µM [O2].

PC-rich *Synechococcus* sp. showed a peak in growth rate under red light of 620 or 660 nm, absorbed by phycocyanin and chlorophyll. Under 2.5 µM [O2] the PE-rich strain showed high growth rates under 530 nm – 660 nm absorbed by phycoerythin, phycocyanin, and chlorophyll; while under 250 µM [O2], the PE-rich strain showed the highest growth rate under green light of 530 nm absorbed by phycoerythrin.



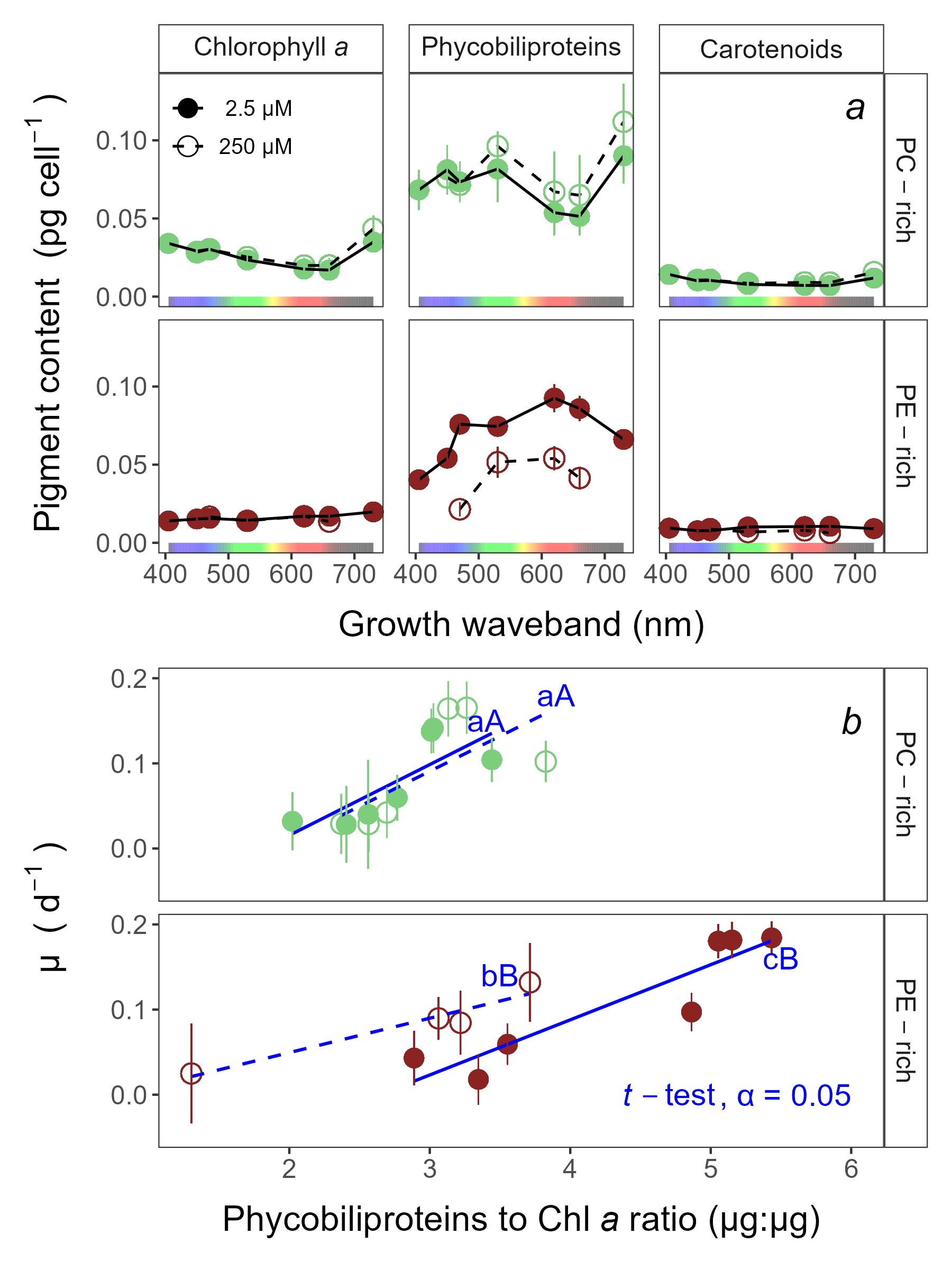
**Fig.** 1: Chlorophyll-specific growth rates (µ; d−1) vs. growth waveband (nm, shaded regions). Growth rates (± SE) were estimated from logistic fits of chlorophyll proxy OD680 – OD720 (ΔOD) vs. elapsed time (Fig. S1), for PC-rich (green circle) and PE-rich (red circle) cultures of *Synechococcus* sp. grown at spectral wavebands of 405, 450, 470, 530, 620, 660, or 730 nm, and [O2] of 250 µM (open symbols and dashed line) or 2.5 µM (closed symbols and solid line).

## Pigment content and pigment ratio across [O2], spectral wavebands, and strains

Fig. 2*a* presents Chlorophyll *a* (Chl *a*), Phycobiliproteins (Phyco), or Carotenoids (Car) content (pg cell −1) vs. growth waveband (nm) for PC-rich and PE-rich cultures of *Synechococcus* sp. grown at spectral wavebands centred at 405, 450, 470, 530, 620, 660, or 730 nm and 250 or 2.5 µM [O2]. Here, we omit data from those PE-rich cultures which showed negligible growth under 405, 450, 730 nm and 250 µM [O2]; and from those PC-rich cultures which showed negligible growth under 405 nm and 250 µM [O2].

Strain, growth waveband, [O2], and their interactions, significantly affected cell-specific Chl *a*, Phycobiliproteins, and Car content (Table S5). For the PC-rich strain, the highest Chl *a*, Phycobiliproteins, and Car contents were recorded after growth under 730 nm. Moreover, the phycobiliproteins content was higher under 250 µM [O2] than under 2.5 µM [O2] for the PC-rich strain. In contrast for PE-rich *Synechococcus* sp. phycobiliproteins content was significantly lower under 250 µM [O2] than under 2.5 µM [O2], with the highest phycobiliproteins content under 620 nm and 2.5 µM [O2].

Chlorophyll-specific growth rates (µ; d−1) show positive linear responses to Phycobiliproteins:Chlorophyll *a* ratio (µg:µg), for both PC-rich and PE-rich *Synechococcus* sp. (Fig. 2*b*), although the relations vary across strain and [O2] (Tab. S10).



**Fig.** 2: Pigment content (pg cell −1) vs. growth waveband (nm) (*a*) and Chlorophyll-specific growth rates (µ; d−1) vs. Phycobiliproteins:Chlorophyll *a* ratio (µg:µg) (*b*) for PC-rich (green circle) and PE-rich (red circle) cultures of *Synechococcus* sp. grown at spectral wavebands of 405, 450, 470, 530, 620, 660, or 730 nm and 250 µM [O2] (open symbols and dashed line) or 2.5 µM [O2] (closed symbols and solid line). Data not presented for those PE-rich cultures which showed negligible growth under 405, 450, 730 nm and 250 µM [O2]; nor for those PC-rich cultures which showed negligible growth under 405 nm and 250 µM [O2]. Blue lines shows linear model fit for data from each strain and [O2] (solid for 2.5 µM [O2] or dashed for 250 µM [O2]) across spectral wavebands. Different blue lowercase letters indicate statistically significant differences between the fit models for different [O2] within a given strain. Different blue uppercase letters indicate statistically significant differences between the fit models for different strains within a given [O2] (*t*-test; *p* < 0.05).

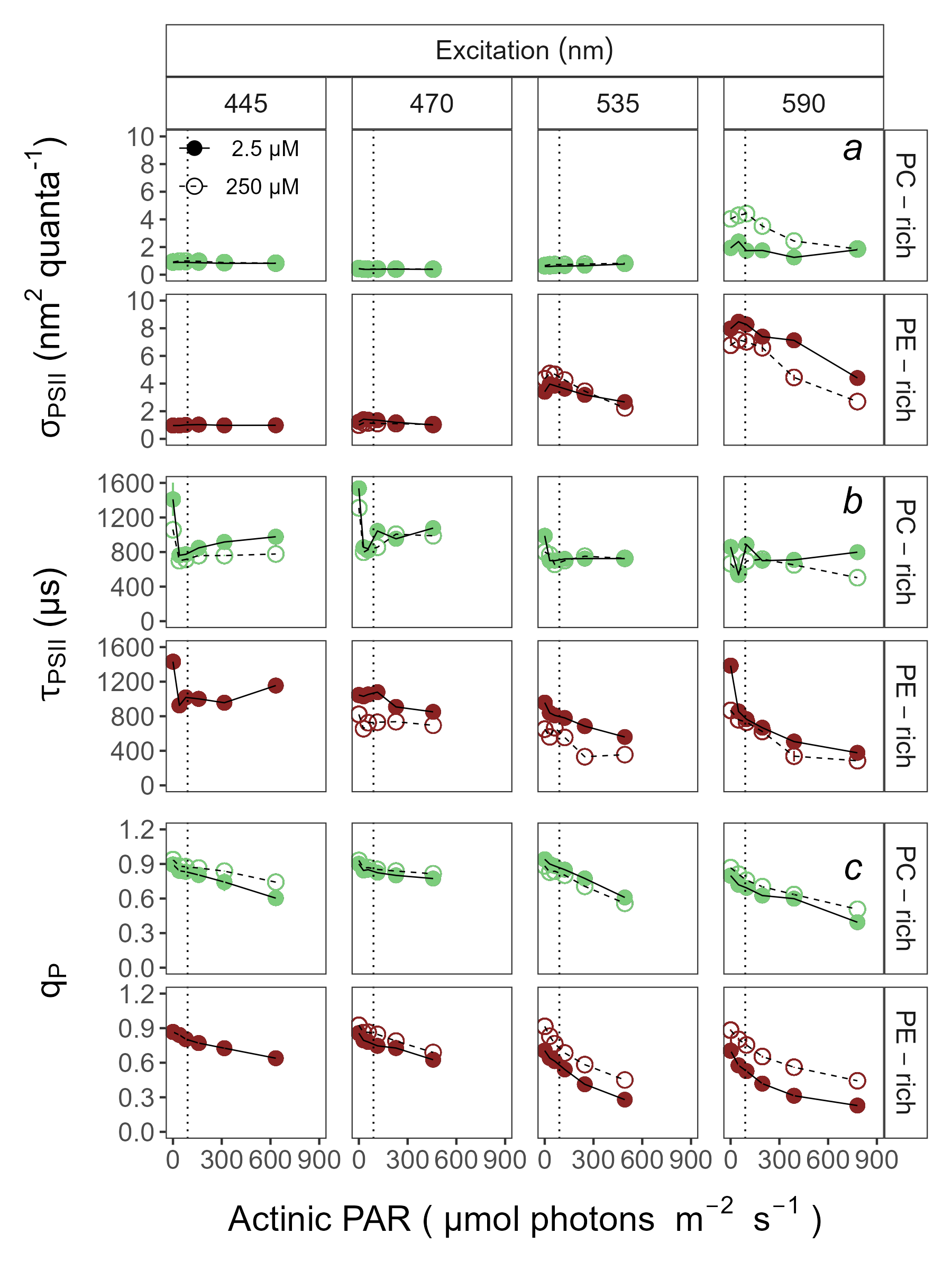
## Effective absorption cross sections, turnover times, and photochemical quenching of PSII across [O2], spectral wavebands, and strains

Light response curves of effective absorption cross section of PSII (σPSII; nm2 quanta−1); turnover time of PSII photochemistry (τPSII; µs); and photochemical quenching coefficient (qP) vs. Actinic PAR (µmol photons m−2s−1) (Fig. 3*a-c*) are shown for PC-rich and PE-rich cultures grown in, and excited by, corresponding wavebands of 450, 470, 530, or 620 nm, at 250 µM or 2.5 µM [O2]. We omit functional data for those PE-rich cultures which showed negligible growth under 405, 450, 730 nm and 250 µM O2; and for those PC-rich cultures which showed negligible growth under 405 nm and 250 µM O2. In the Supplementary materials (Fig S4-S6), we also show the light response curves for all available excitation (Ex445nm, blue; Ex470nm, blue-green; Ex535nm, green; or Ex590nm, orange) and growth waveband (450, 470, 530, or 620 nm) cross-combinations.

σPSII (Fig. 3*a*), as expected, was low and shows little change with increasing actinic light during excitation through chlorophyll at Ex445nm. For the PC-rich strain, under orange excitation at Ex590nm, σPSII showed an initial small increase from darkness to the growth light level, followed by a mild decrease with increasing Actinic PAR, and was higher at 250 µM [O2] compared to 2.5 µM [O2]. For the PE-rich strain, we again see a small increase from darkness to the growth light level, followed by a decrease in σPSII with increasing Actinic PAR. Moreover, for the PE-rich strain σPSII was higher in low [O2] conditions than in high [O2] conditions. Strain, Actinic PAR, and [O2] significantly influenced σPSII under excitation at Ex590nm (Table S6).

For the PC-rich strain, across the excitation wavebands tested, τPSII showed an acceleration (decrease) from darkness to growth light Actinic PAR (Fig. 3*b*), to a plateau of ~ 800 µs. PE-rich strains, on the other hand, showed a progressive acceleration (decrease) with increasing Actinic PAR under excitation at Ex470nm, Ex535nm, or Ex590nm, declining towards ~ 400 µs under Ex590nm. Thus, the PE-rich strain showed more capacity to remove electrons from PSII. τPSII, was generally faster (smaller) for both PC-rich and PE-rich strains under 250 µM [O2]. Strain, Actinic PAR, and [O2] significantly affected τPSII at Ex470nm, Ex535nm and Ex590nm (Table S7).

qP, across the excitation wavebands tested, showed a strong decrease with increasing Actinic PAR (Fig. 3*c*). qP was generally higher for both PC-rich and PE-rich strains under 250 µM [O2]. Strain, Actinic PAR, and [O2] significantly affected qP at Ex470nm, Ex535nm, and Ex590nm (Table S8).



**Fig.** 3: Effective absorption cross section of PSII (σPSII; nm2 quanta−1) (*a*); turnover time of PSII photochemistry (τPSII; µs) (*b*); or photochemical quenching coefficient (qP) (*c*) vs. Actinic PAR (µmol photons m−2s−1). Parameters were estimated using FRRf induction curves with excitation (columns) at Ex445nm, blue; Ex470nm, blue-green; Ex535nm, green; or Ex590nm, orange; for PC-rich (green circle) or PE-rich (red circle) cultures of *Synechococcus* sp. Data show situations in which cultures were excited by, and growing in, corresponding growth wavebands of 450, 470, 530, or 620 nm and 250 µM [O2] (open symbols and dashed line) or 2.5 µM [O2] (closed symbols and solid line). The vertical lines show half diel peak PAR growth light of 90 µmol photons m−2s−1. Data not presented for those PE-rich cultures which showed negligible growth under 405, 450, 730 nm and 250 µM O2; nor for those PC-rich cultures which showed negligible growth under 405 nm and 250 µM O2.

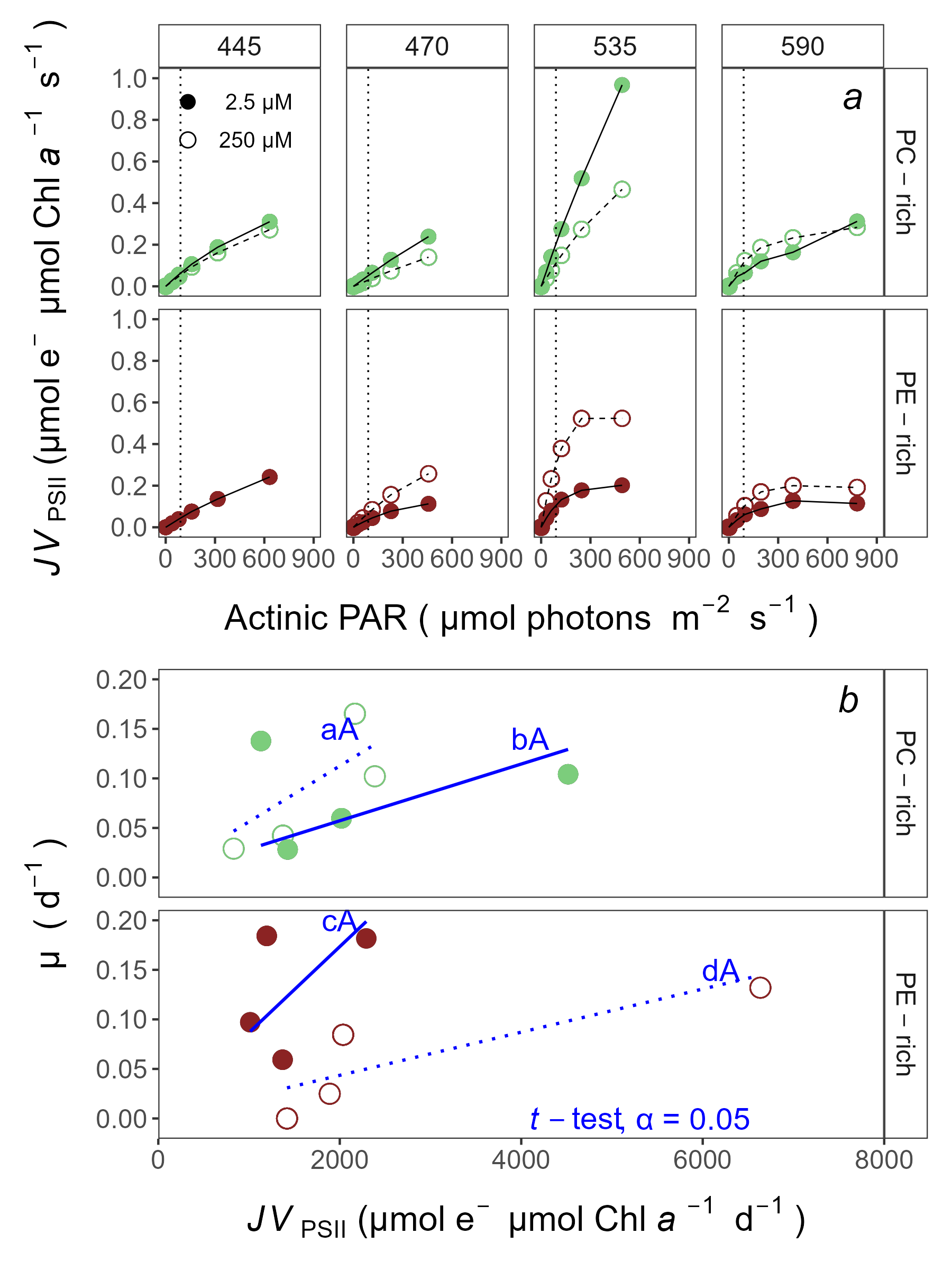
## PSII electron flux across [O2], spectral wavebands, and strains

Light response curves of PSII electron flux (*JV*PSII; µmol e− µmol Chl *a*−1 s−1) vs. Actinic PAR (µmol photons m−2s−1) are shown in Fig. 4*a*. *JV*PSII was estimated using FRRf induction curves with excitation at Ex445nm, Ex470nm, Ex535nm, or Ex590nm, corresponding to growth wavebands of 450, 470, 530, or 620 nm and 250 µM or 2.5 µM [O2]. We did not present data for those PE-rich cultures which showed negligible growth under 405, 450, 730 nm and 250 µM O2; nor for those PC-rich cultures which showed negligible growth under 405 nm and 250 µM O2.

For the PC-rich strain, under all tested excitations (Ex445nm, Ex470nm, Ex535nm, or Ex590nm), *JV*PSII showed an increase with increasing actinic PAR. Under all excitations except Ex590nm we noted that *JV*PSII was higher at 2.5 µM [O2] compared to 250 µM [O2]. On the othet hand, for the PE-rich strain, we see that *JV*PSII under Ex470nm, Ex535nm, or Ex590nm was higher at 250 µM [O2] compared to 2.5 µM [O2]. Moreover, for the PE-rich strain *JV*PSII showed plateau at 90 µmol photons m−2s−1 under Ex535nm, or Ex590nm for both low and hight [O2] conditions. *JV*PSII shows no change between different [O2] with increasing actinic light during excitation through chlorophyll at Ex445nm. Strain, Actinic PAR, and [O2] significantly influenced *JV*PSII under some of the tested excitations (Table S9).

Fig. 4*b* presents linear regressions between chlorophyll-specific growth rates (µ; d−1) and cumulative diel PSII electron flux (*JV*PSII; µmol e− µmol Chl *a*−1 d−1) measured under half diel peak PAR growth light.

µ (d−1), as expected, is positively correlated with *JV*PSII and all presented slopes were significantly different from 0. We have also shown here that the [O2] significantly influences the linear regressions between chlorophyll-specific growth rates and PSII electron flux for both PC-rich and PE-rich strains of *Synechococcus* sp. (*p* < 0.05, Tab. S11). On the other hand, we showed that the relations do not vary for different strains within a given [O2] (*p* > 0.05, Tab. S11).



**Fig.** 4: PSII electron flux (*JV*PSII; µmol e− µmol Chl *a*−1 s−1) vs. Actinic PAR (µmol photons m−2s−1) (*a*). *JV*PSII was estimated using FRRf induction curves with excitation at Ex445nm, blue; Ex470nm, blue-green; Ex535nm, green; or Ex590nm, orange; for PC-rich (green circle) or PE-rich (red circle) cultures of *Synechococcus* sp. Data show situations in which cultures were excited by, and growing in, corresponding growth wavebands of 450, 470, 530, or 620 nm and 250 µM [O2] (open symbols and dashed line) or 2.5 µM [O2] (closed symbols and solid line). *JV*PSII vs. Actinic PAR (µmol photons m−2s−1) was fit with a Harrison and Platt Light Response Curve model (Harrison and Platt 1986), used to estimated *JV*PSII at 90 µmol photons m−2s−1 (vertical dotted lines). Chlorophyll-specific growth rates (µ; d−1) vs. PSII electron flux (*JV*PSII; µmol e− µmol Chl *a*−1 d−1) measured under half (90 µmol photons m−2s−1) of diel peak PAR growth light (*b*). Blue lines (solid for 2.5 µM [O2] or dashed for 250 µM O2) show linear model fit for data from each strain across spectral wavebands. Different blue lowercase letters indicate statistically significant differences between the fit models for different [O2] within a given strain. Different blue uppercase letters indicate statistically significant differences between the fit models for different strains within a given [O2] (*t*-test; *p* < 0.05).

# Discussion

## Growth rate of PC-rich and PE-rich picocyanobacteria across [O2] and spectral wavebands

Picocyanobacteria are a dominant contribution to primary marine production in aquatic ecosystems. What is more, picocyanobacteria from the genus *Synechococcus* is known for its ability to tolerate a wide range of environmental factors (Śliwińska-Wilczewska et al. 2018a; Aguilera et al. 2023) however, the mutualistic influence of [O2] and spectral wavebands on their growth rate and ecophysiology has not yet been sufficiently investigated.

PC-rich and PE-rich *Synechococcus* sp. are entrained in the water column and are therefore often exposed to rapid changes not only in the irradiance intensity but also in the color of light in the mixed layer of the water. Fluctuation in light color may result in photoinactivation of photosystem II beyond counteracting repair rates through protein turnover, thereby leading to photoinhibition and cessation of growth rate (Six et al. 2007). Indeed, we showed that under 250 µM [O2], the PC-rich strain failed to grow under 405 nm, while the PE-rich strain failed to grow under 405, 450, and 730 nm. Failure of growth under 405 & 450 nm is consistent with accelerated photoinactivation of PSII under blue wavebands (Murphy et al. 2017), which increases the cost of growth. In this work we also found that µ showed positive linear responses to both Phycobiliproteins:Chlorophyll *a* ratio, and cumulative diel PSII electron flux (*JV*PSII) for *Synechococcus* sp., although the relations vary across strain and [O2]. The chlorophyll *a* content did not change as dramatically as phycobiliproteins therefore, this means that mostly phycobiliproteins content and the number of electrons flux through the PSII directly affect the growth rate, even when the [O2] is low.

As noted by Wong et al. (2023), vertical structures of phytoplankton communities in the OMZ are not sufficiently explained by top-down predation pressure or factors such as light and/or nutrient limitation and thus, phytoplankton may actually have a higher than expected direct O2 requirement and can be inhibited by low O2 levels. However, in our work we have shown that low oxygen levels do not affect, and sometimes even have a beneficial effect on different *Synechococcus* sp. phenotypes. What is more, historical data link major extinction events to warm climates and oxygen-deficient oceans, suggesting that current anthropogenic activities could lead to widespread oxygen deficiency in the oceans within a thousand years (Breitburg et al. 2018). The PC-rich strain showed generally similar growth rates under tested [O2], while PE-rich strain achieved faster growth rates under low (2.5 µM) than under high (250 µM) [O2]. PE-rich *Synechococcus* are typically found at greater depths, and lower light, than are PC-rich strains (Haverkamp et al. 2009; Śliwińska-Wilczewska et al. 2018a) but we suggest that the PE-rich strains are actually limited to lower light by an interaction between light and full air-saturated [O2]. In lower oxygen waters PE-rich strains are likely able to exploit higher light niches.

## Physiological adaptations of PC-rich and PE-rich picocyanobacteria to [O2] and spectral wavebands

The phenotypes of *Synechococcus* also vary in pigment composition, enabling them to exploit different spectral niches (Moore et al. 1995). With a small diameter of 0.8–2.0 µm, *Synechococcus* sp. possess a high surface-to-volume ratio (Śliwińska-Wilczewska et al. 2018b). This small size minimizes the pigment package effect (Finkel 2001), resulting in high optical absorption efficiency per pigment. This characteristic allows them to thrive deep in the water column (Moore et al. 1995). Although this adaptation is optimal for photon capture, it also increases their susceptibility to light-induced damage (Llabrés and Agustí 2006, 2010). Consequently, *Synechococcus* sp. serves as an intriguing model species for studying photosynthesis and photoacclimation across different oxygen levels and spectral wavebands due to its ecological significance.

*Synechococcus* sp. utilize various pigment-proteins to harvest light energy and protect Photosystem II (PSII) from photodamage. They possess an extra-membrane antenna complex called the phycobilisome, which consists of phycocyanin rods radiating from allophycocyanin core proteins. Core-membrane linker proteins attach the phycobilisome to the thylakoid membrane, transferring harvested light energy to chlorophyll *a* and carotenoids within the PSII complex (Scanlan 2012). In some *Synechococcus* sp., a carotenoids can regulate the connectivity of the phycobilisome to the reaction center, mediating non-photochemical quenching of excitation Kirilovsky (2015). In our work we noted that both carotenoid content (Fig. 2) and the Car to Chl *a* ratio (Fig. Sxxx) did not change under the different [O2]. What is more, for the PE-rich strain, the carotenoids content did not change across tested wavebands. On the other hand, for PC-rich picocyanobacteria, a slight increase in this pigment was recorded at 405 nm and 730 nm. This could indicate that PC-rich *Synechococcus* sp. use carotenoids better to regulate the connectivity of the phycobilisome to the reaction center across different wavebands than PE-rich strain. However, the oxygen levels does not seem to affect these processes in PC-rich and PE-rich *Synechococcus* sp. Alternatively to non-photochemical quenching of excitation, picocyanobacteria can mediate pseudo-cyclic electron flows from PSII back to oxygen through multiple pathways (Grossman et al. 2010; Murphy et al. 2017), thereby controlling feedback inhibition of electron transport and reducing the risk of reactive oxygen species production.

The pattern of σPSII, τPSII, qP, or *JV*PSII versus irradiance can be used to predict the light acclimation status of picocyanobacteria as suggested by Campbell and Oquist (1996). Oxygen is a product of photosynthesis but also have the potential to damage PSII protein subunits. The PsbA protein subunit of PSII is particularly susceptible to irreversible damage leading to the inactivation of the PSII complex (Andersson et al. 1992). The oxygen evolving complex of PSII can be inactivated by a photon in the UV or blue range directly absorbed by the Mn4Ca cluster (Hakala et al. 2005). Here, we showed that σPSII was low and shows little change with increasing actinic light during excitation through chlorophyll at Ex445nm due the fact that in cyanobacteria the number of chlorophyll per PSII is low, and nearly fixed, so the effective absorption cross section for chlorophyll is also low (Xu et al. 2018). τPSII, was generally faster for both PC-rich and PE-rich strains under 250 µM [O2], consistent with the cyanobacterial capacity for electron transport away from PSII to [O2] (Campbell et al. 1999; Hughes et al. 2018), particularly under excess actinic light. As expected, qP was generally higher for both PC-rich and PE-rich strains under 250 µM [O2], since cyanobacteria show strong capacity for electron flow away from PSII to O2, particularly under excess excitation (Campbell et al. 1999; Hughes et al. 2018).

The genus *Synechococcus* is polyphyletic and genetically highly diverse. *Synechococcus* species are phylogenetically divided into several major clusters. Picocyanobacteria that are often found and isolated from marine, brackish and freshwater environments are related to *Synechococcus* cluster 5 (Aguilera et al. 2023). Picocyanobacteria numerically dominate vast tracts of the world’s oceans and contribute a significant proportion of primary production, particularly in oligotrophic regions. The ecological success of picoplanktonic *Synechococcus* sp. suggests they possess sophisticated strategies to respond to variations in their environment. Indeed, it appears that it is the *in situ* community structure of these organisms which underlies this success, with the existence of specific ecotypes or lineages occupying different niches to populate the world’s oceans (Scanlan 2012). Hutchinson’s paradox of the plankton inspired many studies on the mechanisms of species coexistence (Hutchinson 1978). It is commonly believed that picocyanobacteria species can share the light spectrum by specializing in different wavelengths Stomp et al. (2007). Competition models and laboratory experiments have shown that PE-rich picocyanobacteria outperform competitors in green light and PC-rich picocyanobacteria dominate in red light, while both species coexist across the full spectrum (Stomp et al. 2004, 2007). However, in our work, we concluded that not only the color of light, but also the oxygen concentration is crucial in *Synechococcus* sp. populations and that it could reflect extensive diversification of different pigmentation phenotypes into different ecological niches.

# Acknowledgements

We thank Naaman M. Omar for assistance with coding, data analyses and culture maintenance; Miranda Corkum who maintained cultures and trained personnel in culture handling; Laurel Genge, and Carlie Barnhill (Mount Allison students) who assisted with R code.

# Article information

## Data availability statement

Data supporting this study is available on: <https://github.com/FundyPhytoPhys/BalticO2> (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/BalticO2>.

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Conceptualization: SSW, DAC Data curation: SSW Formal analysis: SSW, MS, DAC Funding acquisition: DAC Investigation: SSW Methodology: SSW, MS, DAC Project administration: DAC Resources: DAC Supervision: DAC Validation: SSW, MS, DAC Visualization: SSW Writing – original draft: SSW, MS, DAC

## Competing interests

The authors declare there are no competing interests.

## Funding information

This work was supported by Canada Research Chair in Phytoplankton Ecophysiology (DAC) and Latitude & Light; NSERC of Canada Discovery Grant (DAC).

# Supplementary material

Supplementary data are available with the article at https: //github.com/FundyPhytoPhys/BalticO2.

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