Cyanobacteria of the genus *Synechococcus* play a key role as primary producers and drivers of the global carbon cycle in temperate and tropical oceans. *Synechococcus* use phycobilisomes as photosynthetic light-harvesting antennas. These contain phycocerythrin, a pigment-protein complex specialized for absorption of blue light, which penetrates deep into open ocean water. As light declines with depth, *Synechococcus* photo-acclimate by increasing both the density of photosynthetic membranes and the size of the phycobilisomes. This is achieved with the addition of phycocerythin units, as demonstrated in laboratory studies. In this study, we probed *Synechococcus* populations in an oligotrophic water column habitat at increasing depths. We observed morphological changes and indications for an increase in phycobilin content with increasing depth, in summer stratified *Synechococcus* populations. Such an increase in antenna size is expected to come at the expense of decreased energy transfer efficiency through the antenna, since energy has a longer distance to travel. However, using fluorescence lifetime depth profile measurement approach, which is applied here for the first time, we found that light-harvesting quantum efficiency increased with depth in stratified water column. Calculated phycobilisome fluorescence quantum yields were 3.5% at 70 m and 0.7% at 130 m. Under these conditions, where heat dissipation is expected to be constant, lower fluorescence yields correspond to higher photochemical yields. During winter-mixing conditions, *Synechococcus* present an intermediate state of light harvesting, suggesting an acclimation of cells to the average light regime through the mixing depth (quantum yield of -2%). Given this photo-acclimation strategy, the primary productivity attributed to marine *Synechococcus* should be reconsidered.
Marine photosynthesis by single-celled microorganisms accounts for nearly 50% of global primary productivity. Numerically, the vast majority of primary producers in the oceans are cyanobacteria, the only extant prokaryotic group of oxygenic photoautotrophs. Among these, the two cyanobacterial genera—Prochlorococcus and Synechococcus—are responsible for a significant fraction of primary production, mainly in open ocean waters in subtropical and tropical settings. The basic photosynthetic apparatus in all cyanobacteria consists of two photochemical reaction centers: Photosystem I and Photosystem II. Most cyanobacteria, including Synechococcus that are the focus of our study, possess a supramolecular-light-harvesting antenna coupled mainly to PSI, the Phycobilisome (PBS). Prochlorococcus, however, use membrane internal light-harvesting systems. In Synechococcus, the PBS contains proteins that bind phycocyanin chromophores (PE) absorbing blue light (peak at 497 nm), the wavelength that best penetrates seawater. Owing to this adaptation, this genus specializes in light harvesting in the deeper ocean.

Light regimes through the water column can change dramatically in space and time. Its intensity attenuates exponentially with depth, and its spectrum is narrowed to blue wavelengths. Moreover, the conditions in an open ocean water column vary seasonally. Generally, during summer periods, as the surface warms up and temperature declines monotonically with depth, the water is stratified, and vertical movements of plankton are restrained. Under these conditions, cells inhabiting different water layers acclimate to the available light regime. However, during winter, cooling of the surface drives vertical mixing of the water column. This in turn requires phytoplankton to entrain to a light regime which exposes them to changes on an hour-to-day timescale. Photoacclimation mechanisms cope with light regime changes, which impacts photosynthetic performance and thus productivity.

Among the phytoplankton, cyanobacterial Synechococcus species are known to exhibit extensive photo-acclimation capacities. Known acclimation strategies to low light conditions include increasing both the number and the size of photosynthetic units, a term defining the number of antennae chromophores coupled to a photosystem reaction center. Synechococcus cells under low light will contain a higher number of thylakoid membranes per cell, higher chlorophyll content, and larger phycobilisomes with additional PE units. The plasticity of the Synechococcus is enabled by the position of the PBS antenna in the inter-thylakoid space. However, at the same time, the intermediate chromophore coupling regime determines energy transfer efficiencies that are considered lower than those of thylakoid membrane internal antenna complexes.

Recently, we showed that, in response to low light, the Synechococcus WH8102 strain can improve its phycobilisomes’ light-harvesting efficiency. From a physical point of view, this discovery is surprising, since, with the larger antenna, the absorption cross-section increases but requires the excitation energy to travel longer distances. In land plants, the longer energy migration path was shown to decrease energy transfer rates, as expected according to Forster Resonance Energy transfer calculations. However, in Synechococcus WH8102 the energy transfer rate through the antenna to the reaction centers increased when grown under lower light. We demonstrated that this is achieved by enhanced coupling between chromophores in the phycobilisome.

When light is absorbed in a photosynthetic light-harvesting complex (PBS in the case of Synechococcus), the energy has to migrate through the antenna and reach a reaction center, where photochemical energy conversion takes place. There are three competing pathways that light energy can follow: (i) dissipation through heat; (ii) emission as fluorescence; (iii) photochemical reactions. In the upper water layers, light intensities are high and excess light can be extremely harmful to the cell, due to the generation of reactive oxygen species (ROS). Photosynthetic organisms use a variety of mechanisms to dissipate excess energy, collectively called non-photochemical quenching (NPQ) mechanisms. NPQ levels may vary significantly and therefore influence the heat dissipation rate in surface waters. However, when examining photosynthetic organisms in deeper layers under lower irradiance, heat dissipation is expected to be minimal and constant. In this scenario, changes in the quantum yield of photochemistry ($\Phi_P$) are inversely related to the quantum yield of fluorescence ($\Phi_F$). Comparing the quantum yields of the different processes can be achieved by using fluorescence lifetime measurements. This is a standard method for estimating light-harvesting efficiency in laboratory studies. Using time-correlated single-photon counting (TCSPC) technique to measure fluorescence lifetime in the picosecond time domain, we can quantitatively relate the fluorescence lifetime to the absolute quantum yield of fluorescence ($\Phi_F$).

Results

Recent advances in TCSPC methods have allowed for fluorescence lifetime measurements of photosynthetic communities in situ. These earlier studies tackled the lifetime of chlorophyll in surface water, an abundant and constitutive pigment in all photosynthetic systems. TCSPC has a distinct advantage over fluorescence intensity-based methods, measuring Fv/Fm for example, as it does not depend on the concentration of the measured sample. Here, we specifically address the light energy conversion carried out by Synechococcus, as a function of depth. This is done by pursuing the fluorescence lifetime of Synechococcus phycobilisome systems—excitation at 490 nm, directed at the excitation peak of PE; emission between 515 and 675 nm, capturing only phycobilisome fluorescence and minimizing the contribution of chlorophylls (see samples’ fluorescence spectra in Supplementary Fig. 1). Chrysytophyceae, also contain PE. Yet, these are considerably larger than Synechococcus and were removed from the samples by size-filtration. Their concentrations were negligible, as validated by flow cytometry (Supplementary Fig. 2). Seawater samples from different depths were collected over the course of a year. Flow cytometry allowed us to measure...
**Synechococcus** abundance, size distribution, and level of pigmentation, using standard gating in the light-scatter and orange fluorescence channels. A CTD (conductivity, temperature, depth) system was used to measure the temperature profile of the water column (see "Methods").

Measurements of the average PE and chlorophyll emission intensities for **Synechococcus** populations, at different depths and seasons, are shown in Fig. 1. These values were calculated from flow cytometry, and refer to single cells (analyzed data sample can be found in Supplementary Figs. 3 and 4). During stratification, the fluorescence emission intensity of both PE and chlorophyll increased with depth, indicating a higher concentration of photosynthetic units in cells inhabiting deeper water layers. This result is consistent with previous studies. In contrast, during mixing, the emission of both PE and chlorophyll was uniform across the mixed layer and was similar to the emission intensities of cells found at about 70 m in the stratified water column. Forward scattering (FSC, Fig. 2) and side scattering (SSC, Supplementary Fig. 5) of **Synechococcus**, measured by flow cytometry, are proportional to the diameter of the interrogated cell (i.e., a proxy for cell size) and to the cell's external and internal complexity, respectively. Our results clearly show that during stratification, cells in the upper ~90 m were similar. Below that depth, their size and complexity gradually increased with depth. During mixing, cells at all depths were identical, resembling the small cells found in the upper layers during stratification.

Based on these data we selected representative times and depths for TCSPC measurements—twice during the stratified period (July and August), and twice during the mixing period (November and February). TCSPC measurements were performed on total plankton populations in seawater samples, while **Synechococcus** were targeted according to their unique spectral features. The average fluorescence lifetime of **Synechococcus** phycobilisomes as a function of depth, at the different months of the year, is shown in Fig. 3.

During summer (July, August), the water column in the GoA was stratified, as evident by the gradual decline of the temperature profiles (Fig. 3a, b and Supplementary Fig. 6). In stratified conditions phytoplankton cells are largely confined to the same water layer for a long period of time acclimating to local light conditions. We found that with increasing depth of the water layer, as light intensity attenuates exponentially, fluorescence lifetime shortens (from 0.38 ns down to 0.07 ns). This indicates a significantly faster energy transfer rate in phycobilisomes under low light conditions, which would eventually contribute to higher quantum yields for photochemistry.

In contrast, during the mixing season (November, February), the temperature was fairly uniform across the mixing depth profile (Fig. 3c, d). Under these oceanographic conditions, **Synechococcus** density is relatively uniform through the mixing layer, as cells are actively mixed (Supplementary Fig. 7). Under such actively mixing conditions, the light intensity perceived by the cells varies significantly over short time periods. In November, the mixing depth reached down to ~100 m (Fig. 3c).
The average lifetime of all samples down to that depth was around 0.2 ns, while only in the deepest sample, taken from below the mixed layer (110 m) where light levels are very low, cells exhibited a shorter average lifetime of 0.14 ns. In February, when the water was mixed down to 280 m, the lifetime of all samples was constant, around 0.23 ns (Fig. 3d). This is approximately the lifetime found in samples from a depth of 70 m during summer stratification, which is roughly where the deep-chlorophyll-maximum (DCM) is located during summer. The DCM depth at each cruise can be seen in the chlorophyll fluorescence profiles measured by CTD, which appear in Supplementary Fig. 8.

Fluorescence lifetime can be used to evaluate energy conversion efficiencies. To do so, an estimation of the natural lifetime of a relevant phycobilisome assembly that is detached from photosystems is required. For this purpose, we isolated phycobilisomes from Synechococcus WH8102 cultures. The integrity of the isolated PBS fraction used for the measurements was evaluated by its fluorescence spectra (Supplementary Fig. 9a). Following the methodology presented by Brody and Rabinowitch for chlorophyll, we first evaluated the fluorescence quantum yield $\Phi_f$ through intensity measurements using an integrating sphere spectrometer (Supplementary Fig. 9b). Intact isolated PBS $\Phi_f$ was 0.2. As mentioned, the relation between $\Phi_f$ and lifetime is (Eq. 1): $\Phi_f = \frac{\tau_n}{\tau}$ where $\tau$ is the measured lifetime and $\tau_n$ is the natural lifetime. These isolated Phycobilisomes exhibited an average lifetime of 2.01–2.28 ns and therefore $\tau_n$ is in the range of 10–11.5 ns. Taking the shortest and longest lifetimes measured in the open ocean during stratification (0.38 ns and 0.07 ns at 70 m and 130 m, respectively), gives the following quantum yields of fluorescence: 3.3–3.8% at 70 m and 0.6–0.7% at 130 m. For mixing conditions $\Phi_f = 2–2.3\%$ throughout the depth gradient. Two observations are important with regard to this calculation. The first is that $\Phi_f$ of PBS systems is lower than those measured by Rabinowitch and coworkers for chlorophyll. This is to be expected when comparing a single chromophore to a network of hundreds of intermediately coupled chromophores. The second is that under the light intensities at 70 m or below, NPQ is not expected, $\Phi_f$ is inversely correlated to $\Phi_p$.

**Discussion**

Our results show that the fluorescence lifetime of native *Synechococcus* phycobilisomes varies with depth, and follows a clear trend, which correlates to the conditions in the water column. In a stratified water column, when *Synechococcus* remain at a certain depth for sufficient time to acclimate, the physiological state of the cells at each depth is determined by the ambient light radiation. As depth increases, their size and cellular complexity increase in response to light limiting conditions; the number of photosynthetic units and pigment content increases; yet their fluorescence lifetime becomes shorter. Hence, during stratification, the quantum efficiency of light-harvesting increases as light availability decreases.

During mixing, cells are continuously exposed to varying light regimes, requiring them to optimize their photosynthetic machinery to the average available light intensity perceived. Therefore, sampling during mixing served as a natural control experiment for our stratified water column results. Indeed, lifetime was found to be uniform across the mixing depth. Beyond contrasting the results obtained in stratified water, these results show how *Synechococcus* phycobilisome systems cope with the challenges imposed by mixing. Since light intensities change on a short (hours to days) time scale, they cannot optimize to a specific light regime and adopt a likely average state that can serve light harvesting across the mixed layer. This is an “intermediate state,” which resembles the state optimized for a depth of ~70 m during the stratified season.

A comparison of photo-acclimation mechanisms between natural populations of *Synechococcus* from the stratified water column, in this study, and a previous study done on light-acclimated marine *Synechococcus* strains grown in culture, show similarities. In both cases, PBS fluorescence lifetime was shorter when light intensity was low. Under comparable light intensities, natural populations showed longer fluorescence lifetimes (Fig. 3a, b). For example, based on CTD data, in...
the August dataset (during stratification), under light intensities of 137 μmol photons m⁻² s⁻¹ and 6 μmol photons m⁻² s⁻¹, fluorescence lifetimes values were 0.25 and 0.17 ns, accordingly. Under similar illumination conditions in the lab, PBS fluorescence lifetimes were 0.15 (at 150 μmol photons m⁻² s⁻¹) and 0.1 (at 10 μmol photons m⁻² s⁻¹)20. In both cases, *Synechococcus* acclimated to lower light intensities exhibited shorter lifetimes. The difference in values may be attributed to the diverse population of *Synechococcus* strains in the GoA39, compared to the axenic WH8102 strain grown in culture. In addition, changes in the spectral composition of single-cell fluorescence measured by flow cytometry reported here are comparable to those reported by Six and coworkers44. The photo-acclimation response of the PBS of natural *Synechococcus* populations from the mixed water column are therefore in contrast to both natural stratified *Synechococcus* and laboratory light acclimated *Synechococcus* strains. Going beyond marine *Synechococcus*, studies of freshwater *Synechocystis* further demonstrate the plasticity of PBS fluorescence properties and indicate lifetimes in the range observed here8–47.

Additional photo-acclimation responses of *Synechococcus* cells to the increasing depth, are the increase in cell size and in chlorophyll and phycobilin content. Note that the emission intensity of a pigment or chromophore cannot be used to directly quantify its content and to determine the photosynthetic unit size. Yet, previous studies showed that the phycobilisome size increases with increasing depth and decreasing light intensity44,48. Forward and side scatter of *Synechococcus* showed a depth profile similar to fluorescence lifetime, with a distinct difference between cells residing in the shallow layers and the deeper layers during stratification, and uniform properties across the water column during mixing (Fig. 2 and Supplementary Fig. 5).

Coordinated dynamics of cell size, photosynthetic unit content, and PBS fluorescence lifetime were previously shown in a laboratory study20. Their co-occurrence in our stratified field samples supports the interpretation of the fluorescence lifetime measurements. During mixing fluorescence lifetime had an “intermediate state”, and concurrently forward scatter and side scatter values were low—similar to cells in the shallower layer during stratification.

The ability to manipulate energy transfer efficiency in the phycobilisome is not trivial. Previously, it was known that in response to low light, organisms increase their light-harvesting antennae size21,49,50, therefore increasing the absorption cross-section (absorbing light from a larger surface). However, in a larger antenna, excitation energy must travel a longer distance to reach the reaction centers in the photosystems21. In PBS, where pigment-pigment distances are larger than in plants, the effect of a bigger cross-section is expected to be larger. Indeed, Semi-classical dipole-dipole interaction models of phycobilisome, using FRET (Forster resonance energy transfer), which assume one-dimensional phycobilisome rods, predict such an outcome. A longer antenna rod will lower the energy transfer rate22. Thus, the fact that the energy transfer rate increases in the larger phycobilisome systems of low light acclimated cells is surprising in view of these classical models. However, these results fit with our previous laboratory study using *Synechococcus* WH8102 cultures20, where the enhanced coupling was induced under low blue light conditions. The improved energy transfer rate was shown to be the result of enhanced coupling between the chromophores of PE: Phycourobilin and Phycoerythrobilin. Based on these results, it was suggested that the mechanism is either not purely classical, or that it involves an overlooked inter-rod transfer pathway, possibly due to the higher density of phycobilisome rods under low light conditions. The shift from a predominantly single rod one-dimensional energy transfer to coupled rods that allow multidimensional energy transfer may lead to increased efficiency51.

In principle, a complete picture of the fate of the absorbed energy can be generated from Φε and Φp values (Eq. 2): Φε = Φf - Φp. Φf was calculated from lifetime measurements. Φp is often estimated from variable fluorescence measurements as (Eq. 3); Φf = Φp. However, in cyanobacteria dark Fm values are low52 and Fv values are high due to a contribution of the tail of PBS fluorescence in the chlorophyll measurement channel53. Nevertheless, keeping these limitations in mind we can provide an example of how such a calculation can provide insight. We can use Φp values measured for *Synechococcus* WH810220, using DCMU to get a more accurate reading of Fm. Cultures acclimated to medium or low light conditions, which correlate in the GoA, in summer, to ~60 m and ~120 m. τavg values measured from these depths are 0.17 and 0.38, respectively. Based on these values, the calculated quantum yield of thermal dissipation will be ~80% for 60 m and ~60% for ~120 m. These values are in the range reported by Falkowski and coworkers23 based on chlorophyll lifetime measurements.

Over the past decades, variable chlorophyll fluorescence has been the most sensitive, nondestructive signal detectable in the upper ocean that reflects instantaneous phytoplankton photophysiology34,55. It has been used to estimate the biomass and physiological status of phytoplankton and has fundamentally changed the interpretation of the biological responses to ocean physics34. However, to obtain a complete picture of the energy budget in photosynthetic processes, two of the three competing pathways of absorbed energy (photochemistry, fluorescence, and heat) must be measured. Picosecond fluorescence lifetime measurements can complement variable fluorescence techniques and provide a complete understanding of the fate of absorbed energy. It is also crucial for the development of algorithms for remote sensing techniques (i.e., chlorophyll fluorescence measured by satellites) which are often used to estimate spatial patterns of marine primary production56–58. Such algorithms depend on the comparison with accurate in situ measurements of quantum yields24. Here, we demonstrate the variability in fluorescence quantum yields as a function of depth, highlighting the importance of a depth-profile fluorescence lifetime approach. To reliably estimate the cyanobacterial integrated contribution to photosynthetic activity in the ocean, their dynamics along the water column should be considered. Since our results indicate an increase in efficiency as a function of depth, which was not considered previously, it may suggest an underestimation of *Synechococcus* productivity by current models59,60. With the increase of ocean stratification over the past decades and a similar trend which is expected for the 21st century61,62, incorporating our results into future models may be beneficial for obtaining more precise estimations, accounting for quantum yield changes in response to the water column ambient illumination conditions.

**Methods**

**Water sampling.** Water was sampled at “station A” (29.5° N; 34.95° E; see details in ref. 36), an open sea station in the GoA during 2020 and 2021 (Fig. 1). Depths were chosen in order to capture the different states of the water column during summer: the stratified layer, the DCM, and below the DCM. Same depths were followed during winter mixing. An additional depth of 25 m was added during two cruises in October-November, which further characterized the shallower communities. During July and August, samples for flow cytometry were collected from two depths only, which represented stratified shallow 5 m and the DCM. From each depth, 5 L were sampled and filtered through a 5 μm plankton net. Two samples from each depth were taken for flow cytometry measurements, and the rest were concentrated by filtering through 0.2 μm polycarbonate filters and suspending the phytoplankton in 3 ml seawater from the same depth. Fluorescence lifetime was measured after dark adaptation of 4 h, during which the samples were kept at room temperature.

**Environmental data.** Sea-Bird SBE 19 CTD (Sea-Bird Scientific, Bellevue, WA, USA) was deployed at each cruise to 500 m and recorded data of pressure.
(depth), temperature, salinity, fluorescence, oxygen, and photosynthetically active radiation (PAR).

Flow cytometry. Duplicates of 4 ml from each depth and an additional 4 ml blank (0.22 µm filtered-seawater) for total phytoplankton counts were fixed with 0.25% Glutaraldehyde and 0.01% polyoxamer. Tubes were incubated for 30 min in dark, 4 °C, followed by flash freeze with liquid N2, and stored at −80 °C. Analysis was performed by Attune Nexcel flow cytometer (Thermo Fisher Scientific, Bishop Meadow, Loughborough, UK) by 488 nm laser excitation for 5 min, at a rate of 100 µL min⁻¹. Emission was examined at 574/26 (peak/ half-bandwidth) to detect orange fluorescence of PE, and 695/40 to detect red fluorescence of chlorophyll a. Identification and gating of Synechococcus followed a protocol by Marie et al. (1997).

Fluorescence lifetime. TCSPC technique was used to measure fluorescence decay lifetime at room temperature, in a self-built setup. Excitation was performed with a FluorTime TS-1000 (PTI), with the Fluorescence Decay Analysis Software 1.4, FluorTools, www.fluortools.com. Raw data examples can be found in Supplementary Fig. 10.

Phycobilisome isolation procedure. One liter Synechococcus WH8102 cultures were harvested by centrifugation at 12,000 x g for 7 min. The cells were then resuspended in 0.8 M phosphate buffer at pH 7. Cells were broken using French Press (20,000 PSI, twice). The homogenate was centrifuged for 2 min at 1150 × g, at 4 °C. The supernatant was collected in a new test tube and was centrifuged for 45 min at 18,500 × g at 4 °C. The Pellet was resuspended in 0.8 M phosphate pH 7 and triton X100 was added to give a final concentration of 2% (W/V). The sample was dark incubated for 1 hr at room temp. This was followed by centrifugation for 2.5 h using 147,000 × g at 4 °C. The pellet was then resuspended in 0.8 M phosphate buffer pH 7 and loaded on a sucrose gradient (0.25–2 M), centrifuged overnight using SW41 rotor at 40,000 rpm. The bands were collected, and intact phycobilisome bands were identified according to fluorescence spectra.
