EFFECTS OF TEMPERATURE AND NUTRIENT SUPPLY ON RESOURCE ALLOCATION, PHOTOSYNTHETIC STRATEGY AND METABOLIC RATES OF *SYNECHOCOCCUS* SP.[1]

Running tittle: Temperature-nutrient effects on *Synechococcus*

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Abstract

Temperature and nutrient supply are key factors that control phytoplankton ecophysiology, but their role is commonly investigated in isolation. Their combined effect on resource allocation, photosynthetic strategy and metabolism remain poorly understood. To characterize the photosynthetic strategy and resource allocation under different conditions, we analysed the responses of a marine cyanobacterium (*Synechococcus* PCC 7002) to multiple combinations of temperature and nutrient supply. We measured the abundance of proteins involved in the dark (RuBisCO, RbcL) and light (Photosystem II, PsbA) photosynthetic reactions, the content of chlorophyll *a*, carbon and nitrogen, and the rates of photosynthesis, respiration, and growth. We found that RbcL and PsbA abundance increased with nutrient supply, whereas a temperature-induced increase in PsbA occurred only in nutrient-replete treatments. Low temperature and abundant nutrients caused increased RuBisCO abundance, a pattern we observed also in natural phytoplankton assemblages across a wide latitudinal range. Growth, photosynthesis and respiration increased with temperature only under nutrient-sufficient conditions. These results suggest that nutrient supply exerts a stronger effect than temperature upon both photosynthetic protein abundance and metabolic rates in *Synechococcus* sp. and that the temperature effect on photosynthetic physiology and metabolism is nutrient dependent. The preferential resource allocation into the light instead of the dark reactions of photosynthesis as temperature rises is likely related to the different temperature dependence of dark-reaction enzymatic rates versus photochemistry. These findings contribute to our understanding of the strategies for photosynthetic energy allocation in phytoplankton inhabiting contrasting environments.

Keywords: Activation energy, D1 (PsbA) protein of PSII, Metabolic rates, Photosynthetic

strategy, RuBisCO, Temperature, Nutrient supply.

List of abbreviations: N-limited, nutrient limited, N-replete, nutrient replete, E_a, activation energy, P^C, Carbon-specific photosynthesis, P^{Chla}, Chlorophyll *a*-specific photosynthesis, R^C, Carbon-specific respiration, C:N, carbon to nitrogen ratio, C:Chl*a*, carbon to chlorophyll *a* ratio, POC, particulate organic carbon, PON, particulate organic nitrogen, μ_{max}, maximum growth rate.

Introduction

Rising sea surface temperatures, associated with increasing nutrient limitation in low-latitude, open-ocean regions, and growing anthropogenic eutrophication of the coastal zone represent some of the most pervasive effects of global change in marine ecosystems (Doney et al. 2012). Temperature and nutrient supply play key roles in controlling both resource allocation at the individual level and rates at which materials move through food webs, thus contributing to regulation of ecosystem functioning (Cross et al. 2015). Temperature influences phytoplankton directly through its effect on growth and metabolic rates (Eppley 1972, Chen et al. 2014). This effect is mostly related to kinetic responses such as increasing enzyme and ribosome activity as temperature rises (Geider 1987), which lead to enhanced rates of protein synthesis, light-saturated photosynthesis, and growth (Raven and Geider 1988). Equally important are nutrients, which are used to synthesize essential biomolecules, including the photosynthetic machinery, that sustain biochemical functions. The significance of nutrients lies in the fact that there is often a mismatch between their availability in the environment and the demands from organisms (Cross et al. 2015). Considering that as much as 80% of the global ocean is nutrient limited (Moore et al. 2013), an understanding of how phytoplankton acclimate and adapt to temperature must also consider the role of nutrient supply.

However, the effect of temperature upon phytoplankton metabolic rates and growth has been studied mostly under nutrient-replete conditions. Only recently has the combined effect of these two variables been investigated in the laboratory (Skau et al. 2017, Marañón et al. 2018) and in the field (Lewandowska et al. 2014, Marañón et al. 2014, Morán et al. 2018). These studies suggest that the temperature effect may depend on nutrient availability, such that metabolic rates may be more responsive to temperature when there is a high nutrient supply, which suggests an interactive response between these drivers.

The molecular catalysts of oxygenic photosynthesis, including photosystem II (PSII) and ribulose-1,5-biphosphate carboxylase:oxygenase (RuBisCO), are highly conserved in all photosynthetic organisms (Campbell et al. 2003, Macey et al. 2014) and play a key role in their metabolism and ecophysiology (Li and Campbell 2017). RuBisCO catalyses CO_2 fixation (the dark reactions of photosynthesis) and may be the most abundant protein on Earth (Ellis 1979, Bar-On and Milo 2019). Under saturating light, the catalytic rate of RuBisCO often constrains the rate of photosynthesis because it is inefficient (Erb and Zarzycki 2018) and temperature dependent (Geider 1987). PSII binds chlorophyll (as such contributes to the cellular chlorophyll content) and performs the dual role of absorbing light and catalysing the splitting of water, dictating the rate of the light reactions of photosynthesis, which are considered temperature independent (Geider 1987, Ensminger et al. 2006).

Toseland et al. (2013) showed that the rate of protein synthesis in eukaryotic phytoplankton increases with temperature. Under nutrient-replete conditions, *Synechococcus* sp. is able to regulate photochemistry over a range of increasing temperatures by increasing the abundance of photosynthetic proteins, including PsbA from PSII, which reflects the need to increase photosynthesis as growth rate increases (Mackey

et al. 2013). Young et al. (2015) found that psychrophilic phytoplankton species, to cope with ambient temperatures that are well below the thermal optimum for most enzymes, increase the abundance of RuBisCO but not of PSII. Under nutrient limitation, resource allocation into photosynthetic proteins can become restricted, resulting in lower growth rates (Falkowski et al. 1989, Halsey and Jones 2015). Given that most studies have investigated the effect of temperature or nutrient supply in isolation, their combined effect upon the photosynthetic machinery remains largely unknown.

The elemental composition and stoichiometry of phytoplankton reflects the changing resource allocation into different macromolecular pools (Moore et al. 2013) and is therefore sensitive to variability in temperature and nutrient supply. The ratio between organic carbon and chlorophyll *a* content (C:Chl*a*) is a central variable in phytoplankton ecophysiology (Geider 1987) that shows consistent patterns in response to abiotic factors, such as an increase with increasing irradiance and a decrease with increasing temperature (Maxwell et al. 1995, Geider 1987, Geider et al. 1997, Halsey and Jones 2015). The ratio between carbon and nitrogen (C:N ratio) can also change in response to environmental variability. However, it has been found to remain relatively constant with temperature under nutrient replete conditions in cultures (Spilling et al. 2015, Yvon-Durocher et al. 2015, Skau et al. 2017) and over a range of different nutrient conditions in the field (Yvon-Durocher et al. 2015, Young et al. 2015), where it was not correlated with temperature. While the variability in C:Chl*a* (Maxwell et al. 1995) and C:N (Moreno and Martiny 2018) ratios as a function of temperature or nutrient supply has been well investigated, changes in stoichiometry due to concurrent variability in both these drivers remain unclear.

Cyanobacteria contribute substantially to both phytoplankton biomass and primary production in the marine environment, particularly when nutrients are limiting (Partensky et

al. 1999). *Synecchococcus* spp. are a significant component of this group (Waterbury et al. 1979), being widely distributed throughout coastal and oceanic environments from the Equator to the high latitudes (Huang et al. 2012, Flombaum et al. 2013), which makes it an appropriate microorganism for studying a wide range of contrasting environmental conditions. To elucidate the combined effects of temperature and nutrient supply upon the photosynthetic machinery and metabolism of *Synechococcus* sp. we make use of the experiments described by Marañón et al. (2018), in which nitrogen-limited continuous cultures (at dilution rates 0.1 and 0.3 d^{-1}) were maintained at 4 temperatures over the range 18-30 ºC. In addition, we present the results of a new experiment carried out under nutrient-replete conditions over the same temperature range. For all combinations of temperature and nutrient supply, we assessed the resource allocation to photosynthesis and the photosynthetic strategy of the cells by measuring the abundance of the photosynthetic proteins PsbA (PSII protein D1 precursor) and RbcL (RuBisCO large subunit), together with C:N and C:Chl*a* ratios and the rates of photosynthesis, respiration and growth. To link the patterns observed in laboratory with natural variability in the ocean, we also determined variability in RbcL abundance in phytoplankton assemblages across a wide biogeographic gradient covering tropical, temperate and polar regions. Our main goal is to determine the combined role of nutrient availability and temperature in regulating resource allocation, photosynthetic metabolism and growth of *Synechococcus* sp. In particular, we assess the hypothesis that the effect of temperature on photosynthetic protein abundance and metabolic rates (photosynthesis and respiration) is dependent on nutrient availability.

Materials and Methods

We maintained cultures of the marine cyanobacterium *Synechococcus* PCC 7002 (henceforth referred as *Synechococcus*) growing over a range of temperatures from 18 to 30

ºC under contrasting nutrient supply regimes, from strongly nitrogen-limited (N-limited) continuous growth in chemostats to nutrient-replete (N-replete) exponential growth in semicontinuous batch cultures. Steady-state, N-limited chemostats allow the monitoring of populations that are fully acclimated to chronic nutrient limitation and might be considered as a laboratory homologue of the oligotrophic central gyres. N-replete, semicontinuous batch cultures, in contrast, represent near-optimal conditions that provide a homologue of transient situations at sea when populations sustain fast growth rates (e.g. blooms). The combination of these contrasting experimental settings thus allowed us to characterize photoautotroph metabolism and growth over a wide ecophysiological gradient.

N-limited chemostat cultures

We maintained *Synechococcus* under N-limited, continuous growth in a Sartorius Biostat Bplus bioreactor, as described by Marañón et al. (2018). To ensure nitrogen limitation of growth, we used a modified f/4 medium with a N:P ratio of 10. The nutrient concentration in the incoming medium was 181.18 µmol nitrate $\cdot$ L^{-1} and 18.12 µmol phosphate $\cdot$ L^{-1}. The dilution rates used, which at steady state equal the population growth rate, were 0.1 d^{-1} and 0.3 d^{-1} and the cultures were maintained at four temperatures for each dilution rate, 18, 22, 26 and 30 ºC ± 0.5 ºC, avoiding supraoptimal temperatures (Mackey et al. 2013). The bioreactor was equipped with two vessels of 2 L and the cultures were aerated with natural air pumped through a 0.45 µm nylon filter. Growth-saturating irradiance (200 µmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}, Six et al. 2004) was provided by LED tubes with a 12:12 (light:dark) photoperiod. After an acclimation period of at least 10 days and when the populations had reached steady-state growth, samples were taken for each combination of temperature and dilution rate. We took samples for the determination of elemental composition, metabolic rates (photosynthesis and respiration) and the abundance of

RuBisCO and D1 protein from photosystem II (proteins encoded by *RbcL* and *PsbA* genes, respectively).

N-replete semicontinuous batch cultures

We grew *Synechococcus* in f/4 medium with a nitrate and phosphate concentration of 441 μmol · L^{-1} and 18 μmol · L^{-1}, respectively. Daily transfer to fresh medium was used to maintain the population under N-replete, exponential growth. Growth temperatures were the same as for the N-limited treatments. We calculated the growth rate (μ) from daily measurements of in vivo fluorescence, as the maximum slope of the linear regression between time and the natural logarithm of fluorescence. The cultures were maintained in 2-L borosilicate round flasks with bubbling air pumped through a 0.45 μm nylon filter. The irradiance conditions were the same as described above for the N-limited cultures. After an acclimation period of at least 10 days, we obtained samples for elemental composition, metabolic rates, and RbcL and PsbA abundance.

Chlorophyll a (Chla) and particulate organic matter

In vivo fluorescence was measured daily with a TD-700 Turner fluorometer (Turner Designs, San Jose, CA, USA). We also determined Chl*a* concentration fluorometrically on 5-mL samples filtered through 25-mm diameter GF/F Whatmann filters, stored at -20 ºC and extracted with 90% acetone overnight. Particulate organic carbon (POC) and nitrogen (PON) were determined on duplicate 10-mL samples filtered through pre-combusted 25-mm of diameter GF/F filters and stored at -20 ºC. Filters were dried at room temperature for 48 hours and then analysed with a Carlo Erba Instruments EA 1108 elemental analyser.

Photosynthetic protein analyses

Culture samples (20-300 mL in volume) were filtered onto 0.2-μm polycarbonate filters, which were transferred to cryovials, flash-frozen with liquid nitrogen and stored at -

80 ºC. For protein extraction, 500 µL of denaturation protein extraction buffer was added to each filter (140 mM Tris base, 105 mM Tris-HCl 0.5 mM EDTA, 2% lithium dodecyl sulphate, 10% glycerol, and 0.1 mg · mL^{-1} PefaBloc SC protease inhibitor (Merck, Darmstadt, Germany)). The filters were then flash-frozen in liquid nitrogen and total protein was extracted using 4 rounds of sonication with a Vibra-Cell Ultrasonic Processor with a micro-tip attachment (Sonics and Materials, Newton, CT, USA), as described by Brown et al. (2008). To avoid over-heating, between each round of sonication, samples were refrozen immediately in liquid nitrogen. The total protein concentration of the extracts was determined using the BCA protein assay (Pierce, Thermo Fisher Scientific, Waltham, MA, USA), and the Chl*a* concentration of the extract was measured fluorometrically. The abundance of RuBisCO, here represented as the large subunit encoded by the *RbcL* gene, and the D1 protein, core reaction centre of photosystem II encoded by the *PsbA* gene, was determined by Western Blotting. Total protein extracts (1-2 µg total protein) were separated by SDS-PAGE alongside a series of 3-4 RbcL or PsbA protein standards (Agrisera, Vännäs, Sweden) of known concentration on 1.5-mm NuPAGE Bis-Tris 4-12% acrylamide gradient mini-gels with 1X MES running buffer (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Gels were run in an XCell Sure-Lock Tank (Invitrogen) at 200 V for 35 minutes. The separated proteins were transferred to a polyvinyl difluoride (PVDF) membrane pre-wetted with methanol and equilibrated in 1X NuPAGE Transfer Buffer (Invitrogen) containing 10% methanol. Transfers were run in an XCell blot module (Invitrogen) at 30V for 55 minutes (PsbA) or 70 minutes (RbcL). Blots were probed with polyclonal, global anti-PsbA or anti-RbcL primary antibodies (1:40000 *PsbA*; 1:30000 *RbcL*) (Abcam, Cambrigge, UK) as described by Brown et al. (2008). Blots were developed with Amersham ECL Select Western Blotting Detection Reagent (GE Healthcare Life

213 Sciences, Buckinghamshire, UK) and imaged with a LI-COR C-DiGit blot scanner (LI-
214 COR Biosciences, Cambridge, UK). Band intensities for protein standards and samples
215 were quantified using Image J (Schneider et al. 2012).

216 Protein standard band intensities were plotted as standard curves and used to
217 estimate PsbA and RbcL quantities in the loaded samples. Results were only used when
218 samples fell within the linear range of the loaded standards.

219 The abundance of both RbcL and PsbA were expressed in pmol · (µg total protein)$^{-1}$
220 , pmol · (pmol Chl*a*)$^{-1}$ and as a weight percentage relative to total protein. For the latter,
221 we took into account that *Synechococcus* contains Form 1 RuBisCO with eight equimolar
222 subunits per molecule (RbcL and RbcS). Picomoles of RbcL were converted to µg of RbcL
223 using the molecular weight of 52.159 kDa (UniProt ID Q44176) and µg of RbcS were
224 calculated using equimolar pmol and a molecular weight of 13.212 kDa (UniProt ID
225 Q44178). The D1 core reaction centre of PSII, PsbA, has a molecular weight of 39.711 kDa
226 (UniProt ID B1XM24) and µg of the samples were calculated directly from the pmol
227 quantities measured on the Western Blots. Finally, concentration of both proteins,
228 RuBisCO and PsbA, were expressed as a percentage of the total protein (µg) loaded onto
229 the gel.

230 *In situ RuBisCO abundance*

231 To complement the laboratory experiments we included 65 samples that had been
232 collected from surface waters during four different cruises spanning polar, temperate and
233 tropical latitudes (64˚N to 78˚S), thus covering a wide range of environmental conditions
234 including temperature and nutrient availability: 26 samples from the *RVIB Nathaniel B.*
235 *Palmer* cruise to the Ross Sea (cruise NBP12-01, 72-78°S, 160°W-160°E; see Ryan-Keogh
236 et al. 2017) from 24th December 2011 to 10th February 2012; 16 samples for the *RRS*

Discovery cruises to the subpolar North Atlantic, a spring cruise (cruise D350, 58-63°N, 16-36°W; see Ryan-Keogh et al. 2013) from 28th April to 10th May 2010 and a summer cruise (cruise D354, 56-64°N, 8-42°W; see Ryan-Keogh et al. 2013) from 4th July to 10th August 2010; and 23 samples for the *RRS James Cook* AMT19 cruise (Atlantic Meridional Transect, 50°N to 47°S) from 13th October to 1st December 2009. In all cases, whole seawater was collected from Niskin bottles on a CTD rosette system from 5 metres depth. Samples for protein extraction were collected by filtering 1.0-3.0 L of seawater onto GF/F Whatman filters under low light for ~45 minutes to minimize changes in protein abundance following sampling. Filters were flash-frozen and stored at -80 °C until analysis. RbcL protein abundance, used as a proxy of RuBisCO abundance, was quantified using the techniques described above (Brown et al., 2008). The abundance of RbcL was expressed in pmol (µg total protein)$^{-1}$ using the molecular weight of RbcL as described above.

Metabolic rates

Rates of photosynthesis and respiration were determined with the O_2-evolution technique. Eight gravimetrically-calibrated and acid-washed, borosilicate glass bottles of 30 mL in volume were filled with culture. Two replicate bottles were fixed immediately for initial oxygen concentration and the other six bottles were incubated for 2.5 h in a temperature-controlled chamber. Three bottles were incubated in darkness and the other three were incubated under the same irradiance conditions experienced by the cultures. Dissolved oxygen concentration was measured with the Winkler technique using a potentiometric endpoint. To obtain the metabolic rates in units of carbon, we applied a molar O_2 to CO_2 ratio of 1.4 (Laws 1991).

Carbon-specific photosynthesis (P^C) and respiration (R^C) were calculated by dividing hourly metabolic rates by POC concentration, while chlorophyll *a*-specific

photosynthesis ($P^{Chl\mathit{a}}$) was calculated by dividing the photosynthesis rate by Chl*a* concentration.

Data treatment and statistical analyses

We used linear regression analyses to assess the effect of temperature and nutrient supply upon photosynthetic protein abundance, elemental stoichiometry, and metabolic rates. Normalisation was required to remove the effect of either temperature or nutrient supply and analyse the effect of the other driver in isolation. Normalisation of a given variable was conducted by dividing each value by the mean value for the corresponding nutrient or temperature treatment. Growth rate was used as a common metric for nutrient supply in both N-limited and N-replete cultures. The non-parametric Kruskal-Wallis *H* test was used to assess differences among temperatures within a given nutrient treatment, followed by a Dunn-Bonferroni's post-hoc comparison test to ascertain which temperature treatments differed.

We quantified the effect of temperature on metabolic rates and, for the N-replete treatment, on growth rate by calculating the activation energy (E_a). Ordinary least-squares regression was used to determine the slope ($-E_a$) of the linear relationship between $1/KT$ (where K is Boltzmann's constant and T is temperature in °K) and the natural logarithm of carbon-specific metabolic rate or growth rate. Since there was no differential effect of temperature upon photosynthesis and respiration rates in the 0.1 and 0.3 d^{-1} treatments, in these analyses we pooled together the data from both of the nutrient-limited treatments. Thus, we considered only two nutrient conditions, N-replete and N-limited, obtaining a single value of E_a for each one. All statistical analyses were carried out with SPSS v. 22 and R Studio v. 3.5.1.

Results

Abundance of photosynthetic proteins

The abundance of RuBisCO and PsbA in our experiments, expressed as a percentage of total protein, ranged between 0.3-1.7 and 0.01-0.23%, respectively (Fig. 1), which corresponds to an abundance of 0.05-0.25 pmol · (µg total protein)$^{-1}$ for RbcL and 0.003-0.058 pmol · (µg total protein)$^{-1}$ for PsbA (see Table S1 in Supporting Information). Both proteins increased their abundance from the N-limited treatments to the N-replete one by at least a factor of two. In the N-replete treatment, RuBisCO abundance (Fig. 1a) reached 1.7% at the coldest temperature and values around 1.0% for the other 3 temperatures. In contrast, RuBisCO abundance was lower in the N-limited treatments, with a mean value of 0.45% at 0.1 d^{-1} and 0.36% at 0.3 d^{-1}. PsbA abundance was lower than that of RuBisCO (Fig. 1b) but increased more markedly with increasing nutrient supply, from a mean value of 0.02% at 0.1 d^{-1} N-limited treatment to 0.08 at 0.3 d^{-1} and 0.19 in the N-replete treatment. Irrespective of temperature, PsbA and RuBisCO abundance increased with nutrient-dependent growth rate (R^2 = 0.85, n = 12, $p < 0.01$ and R^2 = 0.58, n = 12, $p < 0.01$, respectively, Table S2).

There was a significant effect of temperature on the abundance of PsbA in the N-replete and 0.3 d^{-1} N-limited treatments (Fig. 1b), as shown by the regression between temperature and normalised PsbA content (R^2 = 0.84, n = 8, $p < 0.01$, Fig. S1). In contrast, temperature did not affect PsbA abundance in the 0.1 d^{-1} N-limited treatment nor did it consistently affect RuBisCO abundance in any of the nutrient treatments. The only exception to this pattern was the N-replete treatment, in which RuBisCO abundance was significantly different among temperatures (χ^2 = 8.82, n = 16, df = 3, p = 0.03), showing significantly higher values at 18 °C (*ca*. 50%) than those of all other temperatures (post-hoc Dunn-Bonferroni's test). Due to the strong effect of nutrient supply on total protein

309 abundance, there was a significant, positive correlation between the abundance of each
310 protein (Spearman's $r = 0.6$, $p < 0.05$, $n = 12$, Fig. 2).

311 PsbA abundance was particularly sensitive to nutrient limitation and as a
312 consequence the PsbA:RbcL ratio in the 0.1 d^{-1} treatment (<0.15) was lower than in the 0.3
313 d^{-1} and N-replete treatments (0.15-0.45, Fig. S2). The PsbA:RbcL ratio increased with
314 temperature in the N-replete treatment, but did not show any consistent pattern with
315 temperature under nutrient limitation.

316 The RbcL to chlorophyll *a* ratio tended to increase with decreasing temperature both
317 in the N-replete treatment and the 0.1 d^{-1} N-limited treatment, while showing no consistent
318 relationship with increasing nutrient supply (Table S1, Fig. S3). The PsbA:Chl*a* ratio
319 showed a comparatively smaller degree of variability, and did not show any clear pattern of
320 response to either temperature or nutrient supply (Fig. S3).

321 In situ data showed that the abundance of RbcL, relative to both total protein and
322 total chlorophyll *a* content, increased markedly with decreasing seawater temperature, an
323 effect that was particularly evident for temperatures below 10 ˚C (Fig. 3). For the ensemble
324 of the 65 samples analysed, RbcL abundances ranged between 0.01-0.12 pmol · (µg total
325 protein)$^{-1}$ and 1.5-111 mmol · (mol Chl*a*)$^{-1}$.

326 *Cellular composition*

327 The molar carbon to nitrogen ratio of particulate organic matter (C:N) ranged
328 between 5 and 13, with the lowest values being measured in the N-replete treatments (Fig.
329 4a). There was a significant effect of nutrient supply on the normalised C:N ratio (R^2 =
330 0.57, $n = 12$, $p < 0.01$, Table S2), whereas temperature explained a smaller amount of
331 variability ($R^2 = 0.42$, $n = 12$, $p < 0.05$, Table S2). C:Chl*a*, which ranged between 39 and
332 215 µg C:µg Chl*a*, tended to decrease with increasing nutrient supply and temperature (Fig.

4b). Regardless of the temperature considered, C:Chl*a* was 50-100% higher in the 0.1 d^{-1} treatment than in the N-replete one. There was also a strong effect of temperature on C:Chl*a*, which, over the 18 to 30 °C range, decreased from 215 to 137 mol:mol at 0.1 d^{-1}, from 134 to 51 at 0.3 d^{-1} and from 165 to 39 in the N-replete treatment, resulting in a significant linear relationship between temperature and normalised C:Chl*a* (R^2 = 0.68, n = 12, $p < 0.01$).

Metabolic rates and growth

P^C increased markedly with increasing nutrient supply (Fig. 5a), taking mean values from 0.02 h^{-1} at 0.1 d^{-1} to 0.03 at 0.3 d^{-1} and 0.09 h^{-1} in the N-replete treatment. Temperature had a strong effect in the N-replete treatment, where P^C increased 2-fold with increasing temperature, from 0.06 h^{-1} at 18 ºC to 0.12 h^{-1} at 30 ºC, but not in the N-limited treatments, where P^C remained largely constant over the assayed temperature range. E_a for photosynthesis was 0.32 eV in the N-replete treatment and 0.02 under N-limited growth, whereas E_a for growth rate under N-replete conditions was 0.49 eV (Table 1, Fig. 6). $P^{Chl\,a}$ had values in the range 1.5-10 µgC · µgChl*a*$^{-1}$ · h^{-1} for the ensemble of all temperature and nutrient supply treatments (Fig. 5b). $P^{Chl\,a}$ took much higher values under N-replete conditions than in the N-limited treatment. After normalising to remove the effect of temperature, nutrient supply explained almost half of the variability in $P^{Chl\,a}$ (R^2 = 0.45, n = 12, $p < 0.05$). $P^{Chl\,a}$ responded stronger to changes in temperature, decreasing by approximately 50% with increasing temperature over the 18 to 30 °C range (R^2 = 0.84, n = 12, $p < 0.01$, Table S2). R^C took values between 0.001 and 0.008 h^{-1} (Fig. 5c) and did not show a clear response to nutrient supply. R^C increased markedly with temperature only in the N-replete treatment (E_a = 1.6), whereas it was relatively constant in both of the N-limited treatments.

Discussion

Variability in photosynthetic protein abundance

Our experimental design serves to quantify the range of variability in key photosynthetic proteins across a relatively wide range of environmental conditions. The abundance of RbcL and PsbA ranged between 0.05-0.25 and 0.003-0.06 pmol · (µg total protein)$^{-1}$, respectively, which corresponds to a relative protein content of 0.3-1.7% for RuBisCO and 0.01-0.2% for PsbA. These ranges coincide with previous reports of protein abundance in both natural communities and cultures. For instance, Losh et al. (2012) investigated the effect of CO_2 and nutrient limitation upon phytoplankton stoichiometry and photophysiology in the California Current and found that the abundance of RbcL ranged between 0.03 and 0.20 pmol · (µg total protein)$^{-1}$, while that of PsbA fell within the range 0.01-0.04 pmol · (µg total protein)$^{-1}$. The abundance of RuBisCO in batch cultures of eight microalgae growing under various conditions of nutrient and CO_2 availability ranged between 0.5-6% (Losh et al. 2013). Higher protein contents were found by Li and Campbell (2017), who assessed the effect of different nutrient regimes and growth irradiances in two diatom species and reported abundances in the range 0.7-3 pmol · (µg total protein)$^{-1}$ for RbcL and 0.04-0.1 pmol · (µg total protein)$^{-1}$ for PsbA.

Effect of temperature and nutrients on RuBisCO and PsbA

Losh et al. (2012, 2013) found that RbcL and PsbA content increased with increasing nutrient supply, whereas Li and Campbell (2017) reported that cells growing under N limitation increased their cellular allocation to RuBisCO and PsbA. Our results agree with those of Losh et al. (2012, 2013), as we measured the highest protein contents in the N-replete treatment, irrespective of temperature. Our results also show a positive relationship, already seen in previous studies, between growth rate and RuBisCO

abundance (Falkowski et al. 1989, Raven 1991, Losh et al. 2012, 2013, Young et al. 2015) and between growth rate and PsbA abundance (Macey et al. 2014, Ryan-Keogh et al. 2017).

In our experiments, temperature had a more modest effect on protein abundance than nutrient supply. Furthermore, the effect of temperature was more noticeable under high nutrient supply. Increasing temperature enhanced the abundance of PsbA under N-replete conditions, but not under N-limitation. These results support our initial hypothesis that the effect of temperature on photosynthetic metabolism is, in turn, dependent on nutritional status. In contrast, increased temperature did not result in enhanced RuBisCO abundance. This pattern may arise because the light reactions catalysed by PSII are temperature independent, whereas dark reactions, such as CO_2-fixation by RuBisCO, are temperature-dependent (Geider 1987). Under high resource supply (N-replete, light-saturated growth), increasing temperature leads to faster RuBisCO turnover and higher CO_2 fixation rates, so additional capacity of the PSII light reactions is required to provide the reductants and energy needed for carbon-fixation (Ensminger et al. 2006). Conversely, under strong nutrient limitation cells can no longer invest in protein catalysts, such that protein abundance and biosynthetic rates become temperature-insensitive (O'Connor et al. 2009, Marañón et al. 2018) and, in the case of our N-limited *Synechococcus* population, photosynthetic rates remain constant with temperature. This, then, would explain the lack of change in PsbA abundance with temperature when nutrients are limiting.

Psychrophilic diatoms invest more resources in RuBisCO when temperatures are suboptimal (Young et al. 2015). These authors found that elevated carbon fixation rates during blooms in polar regions are associated with RuBisCO protein content as high as 17%. In our experiments, we observed a significant increase in RuBisCO abundance at 18

405 ºC (the lowest tested temperature) only in the N-replete treatment. Given that 18 ºC is well
406 below the thermal optimum for both the RuBisCO carboxylase activity (Galmés et al.
407 2013) and for the growth rate of this tropical isolate (Mackey et al. 2013), the increased
408 RuBisCO abundance at this temperature might represent an acclimation response to
409 compensate for its decreased catalytic rate.

410 Although RuBisCO only constitutes a small percentage of total protein N (Macey et
411 al. 2014, Young et al. 2015), similar temperature sensitivities for other photosynthetic and
412 non-photosynthetic enzymes may combine to explain why the increase in RuBisCO
413 abundance was found only under N-replete conditions. Overall, these results suggest a
414 preferential resource allocation into PSII instead of RuBisCO as temperature rises, mostly
415 under N-replete conditions, which also supports the existence of an interactive effect
416 between temperature and nutrients that controls the abundance of these photosynthetic
417 proteins.

418 *In situ RuBisCO variability and phytoplankton photosynthetic strategies*

419 Our measurements of in situ RuBisCO abundance allow us to examine if the
420 responses observed in laboratory monocultures can be extrapolated to multispecific
421 phytoplankton assemblages in the field. Conversely, the patterns identified in the laboratory
422 experiments can illuminate the mechanisms underlying the variability in RuBisCO
423 abundance along a wide biogeographic gradient. The temperature range spanned by the in
424 situ samples (0-27 °C) is much wider than that of the laboratory experiments and, as it
425 covers tropical, temperate and polar regions, is associated with large changes in population
426 species composition and functional traits (Barton et al. 2013). Yet, it is remarkable that the
427 pattern of increased RbcL abundance (relative to both total protein and Chl*a*) associated
428 with cold temperatures was consistent between laboratory and in situ observations. These

results suggest a phytoplankton photosynthetic strategy that is similar across single-taxon acclimation and community acclimation and adaptation, whereby the relative abundance of RuBisCO increases at low temperature to overcome the lower catalytic rates of this temperature-dependent enzyme (Young et al. 2015). This low-temperature strategy, however, implies an increased requirement for nitrogen, which which is consistent with the enhanced RuBisCO abundance being found only in high latitude regions (<10 ºC), which are nitrogen-rich environments (Moore et al. 2013). As temperature increases, phytoplankton invest relatively more resources in the light reactions of photosynthesis (i.e. chlorophyll *a*, PSII) to provide the required energy and reductant for the cell. If nutrients are not limiting, at high temperature the increase in Chl*a* to RuBisCO and PsbA to RuBisCO ratios could reflect the increased need to provide the now more efficient RuBisCO with the required reductant and energy needed for carbon fixation. Where nutrients are limiting at higher temperatures there may also be an increased uncoupling between the light and dark reactions of photosynthesis as energy and reductant is used in nutrient uptake and cellular maintenance rather than carbon fixation (Hughes et al. 2018).

Variability in C:N and C:Chla ratios

The elemental composition of phytoplankton reflects the patterns of resource allocation into subcellular components and constitutes a critical factor that regulates nutrient cycling, primary production and energy transfer through marine food webs (Raven and Geider 1988, Arrigo 2005, Moreno and Martiny 2018). Our results demonstrate that C:N ratio in *Synechococcus* is strongly dependent on nutrient supply, showing lower values associated with increasing growth rates and protein content. In contrast, C:N showed only a slight increase with temperature under N-limited conditions while showing no response to temperature under N-replete growth, as has been shown before for multiple phytoplankton

453 species (Yvon-Durocher et al. 2015).

454 The C:Chl*a* ratio was strongly regulated by both nutrient supply and temperature.
455 Phytoplankton adjust their chlorophyll *a* content in response to nutrient availability because
456 the photosynthetic machinery accounts for a high fraction of cellular nitrogen (Eppley
457 1972, Halsey et al. 2010, Halsey and Jones 2015). Strong nutrient limitation (represented in
458 our experiments by the 0.1 d^{-1} dilution rate) causes a reduction in the synthesis of pigment-
459 protein complexes (including PSII), which ultimately leads to high C:Chl*a* ratios associated
460 with slow growth. The C:Chl*a* ratio also increases with decreasing temperature, irrespective
461 of the nutrient treatment. The inverse relationship between temperature and pigment
462 content is a well-established pattern in phytoplankton and higher plants, which may result
463 from an adaptive strategy to attain a balance between the temperature-dependent dark
464 reactions involved in carbon fixation and the temperature-independent light reactions
465 (Geider 1987). At the molecular level, acclimation to high temperature mimics acclimation
466 to low irradiance, as in both cases light-harvesting capacity and the catalysts of the light
467 reactions of photosynthesis (i.e. PSII) are increased to maintain the supply of energy and
468 reductant to the dark reactions for carbon fixation (Maxwell et al. 1995).

469 *Effect of temperature and nutrients on metabolic rates and growth*

470 As in the case of protein abundance, the interactive effect between temperature and
471 nutrient supply also applied to metabolic rates. Both photosynthesis and respiration
472 increased with temperature only under nutrient-replete conditions, while being largely
473 temperature-independent in the nutrient-limited treatments. The E_a values measured in our
474 N-replete cultures for photosynthesis, respiration and growth rates were within the range of
475 E_a values previously reported for picoplankton (Chen et al. 2014). The estimate of E_a for
476 growth rate (0.49 eV) is higher than the value predicted by the metabolic theory of ecology

for photosynthetic organisms (0.32 eV; Allen et al. 2005), which supports the emerging view that the difference in temperature dependence of growth under nutrient-sufficient conditions between autotrophic and heterotrophic planktonic unicells may be smaller than previously assumed (Chen and Laws 2016, Wang et al. 2018).

Chlorophyll *a*-specific photosynthesis is commonly used to assess the metabolic responses of phytoplankton to environmental drivers, and is a key component in bio-optical models of marine productivity, but the interpretation of its variability is complicated by the fact that CO_2 fixation and Chl*a* content (both expressed per unit of carbon biomass) can change markedly as growth conditions vary. Previous studies have shown that P^{Chla} increases with temperature in several species of unicellular photoautotrophs, including cyanobacteria (Fu et al. 2007, Spilling et al. 2015), although there are also reports showing that it can remain stable or even decrease with increasing temperature (Tang and Vincent 2000). In our experiments, P^{Chla} consistently decreased with temperature in all nutrient supply treatments. One possible explanation is that, as a result of increased intracellular Chl*a* content, cells growing under warmer temperatures experienced a decrease in Chl*a*-specific light absorption (*a**), i.e. an enhanced package effect, as observed before in cultures of cyanobacteria and chlorophytes (Sosik and Mitchell 1994, Yin et al. 2016).

Conclusions

Changes in nutrient supply have a larger effect than temperature on photosynthetic protein abundance and metabolism of *Synechococcus*. The effects of temperature upon the photosynthetic machinery, metabolic rates and biochemical composition are also dependent on nutrient availability. Our results suggest that resource allocation into PSII and chlorophyll *a* (representing the light reactions of photosynthesis) increases with temperature, mainly under nutrient-replete conditions, to balance the presumably enhanced

specific catalytic activity of RuBisCO. Low temperatures together with high nutrient availability cause an increased investment in RuBisCO, a pattern that is observed also in natural phytoplankton assemblages across a wide latitudinal range. The response of photosynthesis and respiration rates of *Synechococcus* to increasing temperature is strong (E_a between 0.3-1.6 eV) only under nutrient-sufficient conditions, not under nutrient limitation. These findings contribute to improve our mechanistic understanding of how the biochemical composition, photophysiology and metabolism of this ubiquitous and biogeochemically relevant marine cyanobacterium responds to environmental variability.

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Author contribution

Author contributions were as follows: C. F.-G, T. S. B., C. M. M. and E. M. designed the study, analysed the data, and wrote the manuscript; C. F.-G., M. P.-L. and N. P. obtained samples and data; all authors commented on the manuscript.

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Tables

Table 1. Slope (-E_a, eV) of the ordinary-least-squares linear regression between 1/KT and the natural logarithm of carbon-specific photosynthesis (P^C) and respiration (R^C) for both nutrient treatments, N-limited and N-replete, and growth rate (μ) of the N-replete treatment. 95 % confidence intervals (CI) are given for each estimate.

Variable	Treatment	-*Ea*	n	95% CI	*p*
P^C	N-limited	-0.02	7	-0.62, 0.59	0.95
P^C	N-replete	-0.32	4	-1.43, 0.79	0.35
R^C	N-limited	0.11	7	-0.23, 0.44	0.45
R^C	N-replete	-1.6	4	-3.92, 0.72	0.10
μ	N-replete	-0.49	4	-0.76, -0.21	0.02

Figures

Fig. 1 Relationship between temperature and the abundance, expressed as percentage of total protein content, of a) both subunits of RuBisCO and b) PSII core reaction center protein D1, PsbA, for each nutrient supply treatment. Nutrient supply conditions ranged from nutrient-limited growth in continuous cultures at two dilution rates (0.1 d^{-1} and 0.3 d^{-1}) to nutrient-replete growth in semi-continuous batch cultures (N-replete).

Fig. 2 Relationship between the abundance of PsbA and RuBisCO, expressed as a percentage of total protein content, under each nutrient treatment (represented by symbols). The four data points for each nutrient treatment correspond to the four assayed temperatures (represented by colours in a grey scale).

Fig. 3 Relationship between temperature and in situ RbcL abundance a) relative to total protein and b) relative to chlorophyll *a* (note Y-axis in logarithmic scale), in samples from three cruises spanning polar, temperate and tropical latitudes (64˚N to 78˚S). Data are binned and averaged every 5°C and bars indicate standard errors. $R^2 = 0.83$, n = 7, $p < 0.01$ and $R^2 = 0.42$, n = 7, $p = 0.12$ for the linear regression between temperature and RbcL:Total Protein or RbcL:Chl*a*, respectively.

Fig. 4 Relationship between temperature and a) carbon to nitrogen ratio (C:N) and b) carbon to chlorophyll *a* ratio (C:Chl*a*) for the three nutrient supply treatments. Bars indicate standard deviation. Data for N-limited cultures taken from Marañón et al. (2018).

761 Fig. 5 Temperature dependence of a) C-specific CO_2 fixation (P^C), b) Chlorophyll *a*-
762 specific CO_2 fixation (P^{Chla}), c) C-specific respiration rate (R^C) under nutrient-limited
763 continuous growth at two different dilution rates (0.1 and 0.3 d^{-1}) and nutrient-replete,
764 exponential growth, and d) growth rate (μ) under nutrient replete conditions. Bars indicate
765 standard deviation. Data for N-limited cultures taken from Marañón et al. (2018).

766

767 Fig. 6 Arrhenius plots for a) Carbon-specific photosynthesis (P^C, h^{-1}) and b) Respiration
768 (R^C, h^{-1}) under N-limited and N-replete conditions and c) growth rate (μ, d^{-1}) under N-
769 replete conditions.

Supplementary material

Fig. S1 Relationship between temperature and normalized PsbA abundance for the 0.3 d^{-1} nitrogen-limited and nutrient-replete (N-replete) treatments. Normalisation was conducted by dividing PsbA abundance by the mean abundance in each nutrient treatment, so that the effect of nutrient supply was removed. Line represents the linear regression relationship (R^2 = 0.84, n = 8, p = 0.001).

Fig. S2 PsbA to RbcL abundance ratio as a function of temperature for each nutrient nutrient supply treatment. Bars represent standard deviations.

Fig. S3 Relationship between temperature and the abundance (relative to chlorophyll *a* content) of a) RuBisCO large subunit, RbcL, and b) PSII core reaction center protein D1, PsbA, for each nutrient supply treatment. Nutrient supply conditions ranged from nutrient-limited growth in continuous cultures at two dilution rates (0.1 d^{-1} and 0.3 d^{-1}) to nutrient-replete growth in semi-continuous batch cultures (N-replete).

Table S1 Abundance of RbcL and PsbA (pmol) standardized by the content of total protein · (µg of total protein) and by the chlorophyll a content (pmol Chla) for each experimental treatment. Mean (n = 2 and n = 4 for the N-limited and N-replete treatments, respectively) and standard deviation values are given.

Table S2 Results of the linear regression analyses with temperature (ºC) and growth rate (d^{-1}) as independent variables (X) and normalised abundance of RuBisCO and PsbA (%), C:N and C:Chl*a* ratios, and chlorophyll *a*-specific CO_2 fixation rate ($P^{Chl\,a}$) as dependent variables (Y). Variables were normalised by dividing them by the mean value for each temperature or nutrient treatment in order to cancel the effect of temperature or nutrient supply, respectively. The coefficient of determination of the linear regression model (R^2), the *p* values, and the number of observations (n) are shown. Also given are the intercept and slopes for each regression and their 95% confidence intervals.