Are temperature sensitivities of *Prochlorococcus* and *Synechococcus* impacted by nutrient availability in the subtropical northwest Pacific?

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Keywords: temperature; activation energy; nutrient enrichment, growth rate, dilution experiment

Running head: Nutrient limitation reduces *Synechococcus* thermal sensitivity

Abstract

Temperature sensitivity of phytoplankton growth rate is crucial for predicting the effect of global warming on oceanic primary productivity and the efficiency of the biological carbon pump. To investigate how nutrient availability affects the temperature sensitivity of phytoplankton growth, we estimated the activation energy (E_a) of two dominant picocyanobacteria (*Prochlorococcus* and *Synechococcus*) in the subtropical northwest Pacific using short-term temperature modulated dilution experiments. We also conducted a meta-analysis on a compiled dataset of picocyanobacteria growth rate estimated by the dilution technique. Our results revealed that the E_a of *Synechococcus* growth rate under in situ nutrient conditions was lower than under nutrient-replete conditions. The growth response of *Synechococcus* to warming could, therefore, be weaker under nutrient-limiting conditions than in nutrient-replete waters. In contrast, E_a values of *Prochlorococcus* growth rate showed no difference between the two nutrient supply scenarios. We also found that the reduced E_a of *Synechococcus* growth was most likely related to the increasing trend of the half-saturation constants for growth with increasing temperature. The temperature sensitivity of half-saturation constants and the level of nutrient limitation can counteract the response of *Synechococcus* growth rate to increasing temperature. Our results highlight the importance of considering nutrient availability when evaluating the responses of phytoplankton growth and primary production to climate warming, especially in the oligotrophic ocean.

Introduction

Temperature sensitivity of phytoplankton growth is crucial for determining how the primary production and marine biogeochemical cycles respond to the projected global warming, which would eventually affect the functioning and service of marine ecosystems (Sarmiento et al. 2004). It has been quantified as Q_{10} (= 1.88) in the Eppley curve (Eppley 1972) or activation energy (E_a) in the Boltzmann-Arrhenius equation (Brown et al. 2004), which describes the direct effect of temperature on metabolic processes yet barely considers the influence of resource availability.

Both Q_{10} and E_a of phytoplankton growth applied in models of ocean biogeochemistry are derived from the laboratory-measured data, most of them coming from batch cultures incubated under sufficient nutrient and light conditions (Eppley 1972; Chen and Laws 2017). Under such ideal conditions, temperature is the primary factor that determines phytoplankton growth. The temperature sensitivity hereby is the potential maximum thermal response of phytoplankton growth mainly determined by cellular enzyme processes. However, in nature, phytoplankton growth is also limited by the availability of light and nutrients (Clarke 2003; O'Connor et al. 2009).

Nutrient is one of the most important resources but often a limiting factor for phytoplankton growth in the open ocean (Moore et al. 2013). It is usually negatively correlated with temperature in the ocean due to the stratification of water column triggered by thermocline, which limits the nutrient supply from the sub-surface to the photic zone (Sarmiento et al. 2004). Global warming is predicted to result in 1–3 °C increases in mean sea surface temperature by the end of this century, which will further enhance the stratification of water columns and exacerbate the decline in nutrient supply in the open ocean (Collins et al. 2013; Behrenfeld et al. 2006). The oligotrophic regions such as subtropical gyres were found to have been expanding during the past decades (Polovina et al.

2008). Phytoplankton will thereby experience a more nutrient-impoverished situation in the warming ocean. Under nutrient-limited conditions, as in the oligotrophic ocean, phytoplankton growth was found to be controlled by nutrient concentrations rather than temperature (Marañón et al. 2014). The thermal response of phytoplankton growth rate could, therefore, be constrained and suppressed by the nutrient limitation in nature (O'Connor et al. 2009; Marañón et al. 2018). Nevertheless, the mechanism underpinning the constraining effect of nutrient availability remains elusive, which hinders a better evaluation and prediction of how the primary production will respond to the projected ocean warming with consequent nutrient impoverishment.

Marañón et al. (2018) have recently found that nutrient limitation suppressed the temperature sensitivities of the metabolic rates of several phytoplankton species, and ascribed it to the temperature-dependent nature of the half-saturation constant (K_n) for phytoplankton growth, a parameter describing the effect of nutrient concentration on phytoplankton growth rate in the Michaelis-Menten or Monod function (Monod 1942; Johnson and Goody 2011). Whilst K_n is usually set to be temperature-independent in most Earth System Models (e.g., Yool et al. 2013). Marañón et al. (2018) used a set of chemostat experiments to simulate the chronic nutrient limitation of oligotrophic ocean, while the real ocean could be more complex as the microbial food web allows regenerated nutrients supply for the growth of phytoplankton and involves other processes such as competition and predation (Banse 2013). Thus, investigations on how nutrient availability affects the thermal response of natural phytoplankton growth at the population and community levels and whether their K_n is temperature-dependent are in critical need and will provide better insights into the effect of global warming on marine primary production, especially in the expanding oligotrophic ocean (Polovina et al. 2008).

In this paper, we aim at exploring the effect of nutrient availability on the thermal response of natural cyanobacterial populations in the oligotrophic subtropical northwest Pacific. The region is particularly important in the global/regional climate system as it is located at the boundary of the Western Pacific Warm Pool and transports heat from low latitudes to high latitudes via the Kuroshio Current (Hu et al. 2015). The sea surface temperature of this region has been observed to be higher and undergoing a faster increase than other subtropical regions partially due to the intensification of the Kuroshio Current during the past decades (Wu et al. 2012). In such a warm region, phytoplankton could be vulnerable to warming as they have adapted to the local conditions with optima close to the environmental temperature (Thomas et al. 2012).

The phytoplankton communities in the oligotrophic subtropical northwest Pacific are dominated by the marine cyanobacteria *Prochlorococcus* and *Synechococcus* (Endo and Suzuki 2019) which are the most abundant phytoplankton and the major contributors to primary productivity in the oligotrophic ocean (Buitenhuis et al. 2012). Their temperature sensitivities have recently been explored in laboratory and field studies (Johnson et al. 2006; Chen et al. 2014; Stawiarski et al. 2016), yet few studies evaluated their thermal response under the influence of nutrient availability. We conducted short-term temperature manipulated dilution experiments to estimate the temperature sensitivity of *Prochlorococcus* and *Synechococcus* growth rates under two nutrient scenarios. We also conducted a meta-analysis on a compiled dataset of picocyanobacterial growth rate estimated by the dilution technique (Landry and Hassett 1982) to test the following hypotheses: 1) the temperature sensitivity of both *Prochlorococcus* and *Synechococcus* would be constrained by the in situ low nutrient concentration; 2) the reduced temperature sensitivity should be ascribed to the temperature-dependent K_n.

Materials and Methods

Dilution experiments in the subtropical northwest Pacific

Experimental set-up. The growth rates of *Synechococcus* and *Prochlorococcus* at three different temperatures were measured by temperature modulated dilution experiments conducted aboard the R/V *Hakuho Maru* (JAMSTEC/ University of Tokyo) in December 2016 in the Kuroshio Current and its adjacent waters in the subtropical northwest Pacific Ocean (Fig. 1). The dilution approach was designed to measure the phytoplankton growth and microzooplankton grazing rates simultaneously via diluting the natural plankton communities with in situ particle-free seawater to certain proportions and incubating them for one day (Landry and Hassett 1982). The rates were calculated based on the linear relationship of dilution factors and net growth rates by assuming that the growth rate of phytoplankton is not affected by the dilution treatments and the grazing rate of microzooplankton is proportional to the dilution factors (the proportion of original seawater). To measure the rates at different temperatures, the prescribed mixtures of plankton communities were incubated at designated temperatures: in situ surface temperature (T), T-4°C, and T+4°C. At each station, we used an acid-washed plastic bucket to collect the surface seawater and then gently transferred them into two 20-litre polycarbonate carboys.

The dilution experiments followed the "two-points" dilution technique described in Landry et al. (2011), which has been proven as accurate as the traditional dilution approach with a full dilution gradient and is also reliable when nonlinear grazing response occurs (Chen 2015; Morison and Menden-Deuer 2017). Two dilution levels (25% and 100% of unfiltered seawater) were set up with duplicate bottles for each level. In each dilution experiment, 1.8 L particle-free seawater prepared by filtering the seawater through a 0.22 μm pore-size filter capsule (Pall Corporation) was added into two 2.4 L polycarbonate bottles. The bottles were subsequently filled with the natural unfiltered seawater to their full capacity

to achieve the 25% dilution level. This unfiltered seawater was pre-screened through a 200 μm mesh to remove mesozooplankton. Another two 2.4 L polycarbonate bottles were filled with pre-screened unfiltered natural seawater to obtain the 100% dilution treatment. Nutrients (NH_4^+: 0.5 μM, PO_4^{3-}: 0.03 μM, Fe^{3+}: 1.0 nM; Mn^{2+}: 0.1 nM in final concentrations) were added to the four bottles to ensure no nutrient limitation. As the phytoplankton communities were dominated by picocyanobacteria, the nitrogen was added as NH_4^+ instead of NO_3^- which cannot be utilized by most *Prochlorococcus* strains (Landry et al. 1995; 2011; Moore et al. 2002). To estimate the in situ phytoplankton growth rate, which could be limited by the ambient nutrient concentrations, two extra bottles filled with pre-screened unfiltered seawater without adding nutrient were prepared as controls. All bottles were tightly capped and put into on-deck incubators with designated temperatures for one day. The in situ temperature was maintained by running surface seawater. The other two temperatures were maintained by two temperature controllers (EYELA CA-1100 and CTP-3000). Neutral density plastic film was used to cover all bottles to imitate the in situ light conditions. The carboys, bottles, filters and silicon tubing used in the experiments were sequentially washed with 10% HCl, deionized water, Milli-Q water and in situ seawater before each experiment.

Nutrients and phytoplankton analyses. Samples for determining inorganic nutrients were taken, frozen at -80 °C immediately, stored in -20 °C freezer, and analysed by a QuAAtro autoanalyzer (BL TEC K.K., Osaka, Japan) with certified reference material (Aoyama et al., 2012). The detection limits for NO_2^-, NO_3^-, and NH_4^+ are 0.01 μM, 0.05 μM, and 0.01 μM, respectively. For Chl *a* analysis, seawater (2.4 L) was filtered onto GF/F filters (Whatman) under low vacuum, stored in -80 °C freezer, and analysed by ultra-high performance liquid chromatography (UHPLC) according to the method of Suzuki et al. (2015).

At the beginning and the end of each experiment, samples for flow cytometric (FCM) analyses were taken to measure the initial and final cell abundances of *Synechococcus* and *Prochlorococcus*. The samples (2 mL seawater) were fixed with 0.2% buffered paraformaldehyde (*v*/*v*, final concentration), frozen in liquid nitrogen immediately, and stored in -80 °C freezer until analysis. We used a Becton-Dickson FACSCalibur flow cytometer equipped with an air-cooled argon laser (488 nm) to enumerated the cell abundances of *Synechococcus* and *Prochlorococcus*. The *Prochlorococcus* could be detected by the side scatter (SSC) and red auto-fluorescence emitted by Chl *a* at 680 nm, while the *Synechococcus* could be distinguished from *Prochlorococcus* because of their particular orange auto-fluorescence emitted by phycoerythrin at 575 nm (Olson et al. 1993). To normalize and calibrate the fluorescence and light scattering signals, fluorescent beads (1 μm, Polysciences, Inc.) were added to every sample as an internal standard. Aliquots (600 μL) were run for 2 or 3 minutes on the flow cytometer at a calibrated flow rate (~56 μL min^{-1}) and the raw data were analysed using WinMDI software 2.9 (Joseph Trotter, Scripps Research Institute, La Jolla, CA, USA).

Growth rates estimates. The growth rates of *Synechococcus* and *Prochlorococcus* under nutrient-replete conditions (μ_n, d^{-1}) and microzooplankton grazing rates (m, d^{-1}) were estimated according to Landry et al. (2011). Assuming exponential growth for phytoplankton growth in each bottle, the net growth rate (k, d^{-1}) was calculated as $k = 1/t \ln (P_i/(d_i \times P_0))$, where P_i is the final cell abundance of *Synechococcus* or *Prochlorococcus* in the i^{th} treatment bottle after incubation, d_i is the dilution factor of i^{th} treatment (25% or 100% of unfiltered seawater), P_0 is the initial cell abundance of *Synechococcus* or *Prochlorococcus*, and t is the incubation time (1 day). For the undiluted seawater treatment with nutrient enrichment, the net rate of changes in the cell abundance of *Synechococcus* or *Prochlorococcus* (k_n) is $k_n = \mu_n - m$. Assuming the same growth rate in the diluted treatment and a linear decrease in grazing

mortality with dilution, the net growth rate in the diluted bottle (k_d) is $k_d = \mu_n - d_i \times m$, in which d_i is the fraction of unfiltered seawater (25%). The two equations are solved for the m and μ_n: $m = (k_d - k_n)/(1 - d_i)$ and $\mu_n = m + k_n$. The instantaneous growth rates for *Synechococcus* or *Prochlorococcus* under in situ nutrient condition (μ_0, d^{-1}) were calculated based on the net growth rate in the bottles without nutrient addition (k_0) and the mortality rate induced by microzooplankton grazing: $\mu_0 = k_0 + m$.

Activation energy estimates. The temperature sensitivities of *Synechococcus* and *Prochlorococcus* growth rate were quantified as E_a based on the Boltzmann-Arrhenius equation (Brown et al. 2004):

$$\mu = \mu_c e^{-E_a/k_b T} \qquad (1)$$

where μ is the growth rate, E_a is the activation energy (eV) describing how fast the rates respond to the temperature increase, T is the absolute temperature (K), μ_c is a normalization constant, and k_b is the Boltzmann's constant (8.62×10^{-5} eV K^{-1}). As the Boltzmann-Arrhenius equation is usually used in the physiological temperature range, the rates above optimal temperature are usually removed before fitting the equation (Liu et al. 2019). In the current study, we removed the rates at T+4°C if they were lower than those at T as they could be the rates above the optimal temperature. The data showing no increasing trend were also not included in the calculation. The mean E_a of *Synechococcus* or *Prochlorococcus* growth rates for all stations was estimated using a linear mixed effects model which allows random variations of both intercept and slope (Bates et al. 2014). The model of *Synechococcus* or *Prochlorococcus* growth rates treating stations as random effects associated with E_a was described as follows:

$$ln\mu_{i,j} = (ln\mu_c + \theta_{ri}) + \frac{E_a + \theta_{Ea}i}{k_b}\left(\frac{1}{T_c} - \frac{1}{T_{i,i}}\right) + \varepsilon_{i,j} \qquad (2)$$

where $\mu_{i,j}$ is the growth rate of *Synechococcus* or *Prochlorococcus* at j^{th} temperature $T_{i,j}$ (K) at i^{th} station (μ_n or μ_0), μ_c is the normalized rate at reference temperature T_c (288 K), θ_{ri} and

211 $\theta_{E_a i}$ are random deviations from intercept ($ln\mu_c$) and slope (E_a), respectively. $\varepsilon_{i,j}$ is the j^{th}
212 residual in the i^{th} group. We assumed that the activation energy (E_a) of μ_n should not be
213 affected by nutrient availability, whilst the E_a of μ_0 in the *Eq. 2* will yield the apparent
214 activation energy (E; eV). The difference between E_a and E was tested by adding a factor
215 variable (with and without nutrient addition) to *Eq. 2* with the input of both μ_n and μ_0. The
216 linear mixed effects model was implemented by "*lmer*" in R package "*lme4*" (Bates et al.
217 2014). To gauge the goodness of the fit for the model, conditional R^2 and marginal R^2 were
218 calculated using "*r.squaredGLMM*" in the R package "*MuMIn*" (Nakagawa and Schielzeth
219 2013).

220 *The effect of temperature on K_n in temperature modulated experiments.* The
221 relationship between phytoplankton growth rate and nutrient concentration is usually
222 described as the Michaelis-Menten or Monod function:

223 $$\mu=\mu_{max}\frac{N}{N+K_n} \qquad (3)$$

224 in which μ is the growth rate, μ_{max} is the temperature-dependent maximum growth rate, N is
225 the nutrient concentration, and K_n is the half-saturation constant which is the nutrient
226 concentration when the growth rate is $\mu_{max}/2$. The K_n values of *Synechococcus* and
227 *Prochlorococcus* growth rate under different temperatures were estimated using the
228 corresponding pairs of μ_n and μ_0. The μ_n estimated under nutrient-enriched conditions in the
229 dilution experiments should be equal to μ_{max} in Eq. *3*, while the instantaneous growth rates
230 (μ_0) could be limited by the nutrient concentration in our study region. K_n could be calculated
231 using the formula: $K_n = (\mu_n/\mu_0 \times N)$-$N$, where N is the concentrations of limiting nutrient. In
232 the oligotrophic ocean, N is difficult to quantify due to the extremely low concentration. It is
233 more complicated to determine N in our experiments because the regenerated nutrient from
234 remineralization processes should be included. The regenerated nutrient could be the main
235 nutrient source of *Synechococcus* and *Prochlorococcus*, especially in the nutrient-depleted

236 waters. As the nutrient concentration among the stations would not vary a lot due to their
237 close locations and their small values (Fig.1), K_n should be proportional to the ratio of μ_n/μ_0
238 in our study based on the formula above. Thus, instead of calculating K_n, we used the ratio of
239 μ_n/μ_0 to represent K_n and the intensity of nutrient limitation. In fact, μ_n/μ_0 (generally
240 expressed as μ_0/μ_n) is widely used as a nutrient limitation index of phytoplankton in dilution
241 experiments (Landry et al. 1995). The relationship between the μ_n/μ_0 and temperature can
242 reflect the effect of temperature on K_n.

243 ***Meta-analysis on a compiled field dataset***

244 We extended a published dataset of Chen et al. (2014) which consisted of
245 *Prochlorococcus* and *Synechococcus* growth rates in a variety of regions estimated by the
246 dilution technique (Supplementary dataset). Using the same approach as in our experiments,
247 the dataset included the instantaneous growth rate (μ_0) and nutrient-enriched growth rate (μ_n).
248 Most of the nitrate concentrations in the dataset were analysed using the method of Parsons
249 (2013). Only data from experiments in the surface waters were used and the corresponding
250 photosynthetically active radiation (PAR, mol photons m^{-2} d^{-1}) data were extracted from the
251 Goddard Earth Sciences Data and Information Services (http://disc.sci.gsfc.nasa. gov/). In
252 total, 99 and 243 growth rate estimates of *Prochlorococcus* and *Synechococcus* were included
253 in this dataset.

254 The apparent activation energy (E) of *Prochlorococcus* and *Synechococcus* growth
255 rates were estimated using the corresponding instantaneous growth rates (μ_0) according to *Eq.*
256 *1*:

257 $$\ln\mu_0 = \ln\mu_{c1} + \frac{\mathrm{E}}{k_b}\left(\frac{1}{T_c} - \frac{1}{T}\right) \qquad (4)$$

258 where μ_{c1} is the normalized rate at reference temperature T_c (288 K), T is the temperature (K)
259 corresponding with μ_0.The Ordinary Least Squares (OLS) regression was used for the
260 calculation and performed with the function "*lm*" in R.

The E_a was estimated based on Eq. *5* that teases out the effects of light and nutrient. We used General Additive Models (GAMs), which uses nonparametric smooth functions (*s*, thin plate regression splines) to describe the effects of light and nutrient:

$$\ln\mu_0 = \ln\mu_{c2} + \frac{E_a}{k_b}\left(\frac{1}{T_c} - \frac{1}{T}\right) + s(PAR) + s(\ln(N)) \qquad (5)$$

where μ_0 is instantaneous growth rates used in the Eq. *4*, μ_{c2} is the normalization constant, *N* is the nitrate concentration, *PAR* is the photosynthetically active radiation, other symbols are the same with *Eq. 2*. *N* was log-transformed before GAMs analysis to follow the quasi-normal distribution. The GAMs analysis was implemented by R function "*gam*" in the package "*mgcv*" (Wood 2006).

In addition, we estimated the temperature sensitivity of K_n for phytoplankton growth and explored its effect on the thermal response of growth rate using a nonlinear model combining the effects of temperature, nutrient and PAR on growth rate:

$$\mu_0 = \mu_{c3} e^{\frac{E_a}{k_b}\left(\frac{1}{T_c} - \frac{1}{T}\right)} \frac{N}{N + k_0 e^{\frac{E_k}{k_b}\left(\frac{1}{T_c} - \frac{1}{T}\right)}} \left(1 - e^{\frac{-\alpha_I\, PAR}{\mu_c}}\right) \quad (6)$$

in which k_0 is the normalized half-saturation constant at T_c, μ_{c3} is the normalization constant, E_k is the activation energy of half-saturation constant K_n, α_I is the light affinity, other symbols are the same in *Eq. 2* and *3*. The parameters were fitted using the R function "*nls*". All statistical analyses were implemented using R 3.4.3 (R core Team, 2017).

Results

Affected by the Kuroshio Current, surface waters of the study region were characterized by high temperature (~25 °C) and extremely low nutrient with NO_3^- and PO_4^{3-} concentrations below the detection of quantification (Table 1). Chl *a* concentrations were low with a range of 0.17–0.32 μg L^{-1}. *Prochlorococcus* abundance averaged 84100 cells mL^{-1},

284 which was 10 times higher than average *Synechococcus* abundance (Table 1, Supplementary
285 Information Fig. S1).
286 The effect of nutrient enrichment on *Prochlorococcus* growth was marginal, as their
287 growth rates showed no difference between nutrient enrichment and controls without adding
288 nutrients at three different temperatures (paired *t*-test, $p > 0.05$; Fig. 2). The growth rate of
289 *Synechococcus* was not significantly affected by the nutrient addition at both the lowest and
290 the in situ temperature (paired *t*-test, $p > 0.05$; Fig. 2). In contrast, under warming condition,
291 the growth rate of *Synechococcus* increased significantly when nutrients were added (paired
292 *t*-test, $p < 0.05$; Fig. 2).
293 The growth rate of *Prochlorococcus* and *Synechococcus* increased with increasing
294 temperature under both natural and nutrient replete conditions at most stations (Fig. 3). The
295 growth rate of *Synechococcus* increased in the treatment of 4 °C warming at all stations,
296 while that of *Prochlorococcus* increased under warming condition at only two stations (Sta.
297 RM1 and RM2, Fig. 3). At other stations, warming treatment did not increase the
298 *Prochlorococcus* growth rate. Under the ambient nutrient condition, the apparent activation
299 energy (*E*) of *Prochlorococcus* and *Synechococcus* growth rates was 1.36 ± 0.59 eV and 1.19
300 ± 0.20 eV, respectively (Table 2, Figs. 3, 4). Under the nutrient-enriched condition, E_a of
301 *Prochlorococcus* growth (1.02 ± 0.33 eV) showed no difference with the corresponding *E*
302 value ($p > 0.05$; Table 2, Fig. 3, 4), suggesting that *Prochlorococcus* growth was never
303 nutrient-limited (Fig 2a, Table 1). In comparison, E_a of *Synechococcus* growth was
304 significantly higher than the corresponding *E* value (E_a =1.80 ± 0.29 eV, $p = 0.021 < 0.05$;
305 Table 2, Fig. 3, 4).
306 Nearly all the μ_n/μ_0 ratios of *Prochlorococcus* were around 1, which demonstrated
307 again that the growth rate of *Prochlorococcus* was not limited by ambient nutrient
308 concentrations in our study region and hindered our further exploration on the relationship

between K_n for *Prochlorococcus* and temperature. In contrast, the μ_n/μ_0 ratios of *Synechococcus* were positively correlated with temperature (spearman $R = 0.52$, $p < 0.05$), which suggested that K_n of *Synechococcus* growth increased with increasing temperature (Fig. 5).

In the compiled dataset, the GAMs explained 25% and 30% of the variability of *Prochlorococcus* and *Synechococcus* growth rates, respectively. After controlling the effects of nutrient and light availability, E_a values of *Prochlorococcus* and *Synechococcus* growth rates were 1.95 ± 0.83 eV ($p = 0.069$) and 0.63 ± 0.07 eV ($p < 0.001$), respectively, which were significantly higher than their corresponding E (0.17 ± 0.16 eV and 0.32 ± 0.05 eV, respectively, Fig 6a, d). The growth rates of both *Prochlorococcus* and *Synechococcus* increased with nutrient concentrations but were invariant with light (Fig. 6). Using *Eq. 5*, the nonlinear regression model explained 17% of the variability of *Synechococcus* growth rate, while the model for *Prochlorococcus* could not converge due to the insufficiency of data. E_a of *Synechococcus* growth rate estimated by this model was 0.49 ± 0.10 eV ($p < 0.001$, Supplementary Information Table S1), which was consistent with the result of GAMs. The estimation for the activation energy of K_n was also significant with a value of 0.08 ± 0.04 eV ($p < 0.05$). As the growth rate of *Synechococcus* was not affected by light (Fig. 6b), the estimation for parameter α_I was insignificant ($p = 0.311 > 0.05$).

Discussion

A comprehensive understanding of the interactive effects of temperature and resource availability on phytoplankton growth can provide deeper insights into how marine primary production and biogeochemical cycles respond to climate changes. Our study adds knowledge to the effect of nutrient limitation on the thermal response of natural cyanobacterial population growth in the oligotrophic ocean, with strong implications relevant

to the response of phytoplankton to the projected ocean warming with subsequent intensification of nutrient impoverishment, especially in the subtropical regions.

Thermal responses of Prochlorococcus and Synechococcus growth rate

Temperature plays a vital role in determining the global distribution and community structure of *Prochlorococcus* and *Synechococcus* (Partensky et al. 1999). *Prochlorococcus* are present to the full extent of the photic zone in a 40 °N-40 °S latitude band and absent at temperature below 15°C. Despite often co-occurring with *Prochlorococcus*, *Synechococcus* have a wider geographical distribution ranging from polar to equatorial waters and are more abundant in the nutrient-replete surface waters (Partensky et al. 1999; Flombaum et al. 2013). Their diverse ecotypes/genotypes also show different spatial patterns, which is resulted from their thermal preference and adaption (Johnson et al. 2006). The HLII ecotype of *Prochlorococcus* and clade II and III of *Synechococcus* may be the dominant groups in the surface (sub)tropical waters, such as our study region (Zwirglmaier et al. 2008). Although the effect of temperature on *Prochlorococcus* and *Synechococcus* abundance has been explored (Flombaum et al. 2013), to the best of our knowledge, there were no previous studies estimating the thermal response of the *Prochlorococcus* and *Synechococcus* growth based on in situ measurements in the subtropical oligotrophic waters. Although there are potential problems associated with the short-term temperature manipulated experiments used in our study, for instance, elevating temperatures could artificially impose a 'thermal shock' to the plankton, we prudently designed the experimental temperature to ensure that they did not deviate too much from the ambient temperature.

The sea surface temperature in this region is relatively high (~ 25 °C). However, artificially elevating temperature still stimulates the growth of *Synechococcus* at all experimental stations (Fig. 3a), which indicates that the optimal temperature for *Synechococcus* growth is still higher than the ambient temperature. The growth of

359 *Prochlorococcus* also increased with artificially elevated temperature, but this trend was only
360 found at two stations (Fig. 3b). The results of other four stations suggested that the optima for
361 *Prochlorococcus* growth are close to the ambient temperature, which supported previous
362 study stating that (sub)tropical phytoplankton strains have optima close to environmental
363 temperature and are well-adapted to the local temperature regimes (Thomas et al. 2012). In
364 comparison, *Synechococcus* are more plastic in their thermal adaption and have higher
365 optima than *Prochlorococcus* in the study region. Our results are in line with previous studies
366 on laboratory cultures, demonstrating that the optimal temperatures of many *Prochlorococcus*
367 strains were lower than the *Synechococcus* isolated from similar latitudinal ranges
368 (Stawiarski et al. 2016). Therefore, without considering other factors, we speculate that the
369 projected rising temperature might have a stronger and more deleterious effect on
370 *Prochlorococcus* but be more favourable to *Synechococcus* in the subtropical waters.
371 However, the growth of *Synechococcus* could be more vulnerable to the gradually
372 impoverished nutrient concentration following the increase of sea surface temperature
373 particularly in the subtropical ocean gyre. We found that the ambient nutrient concentration
374 in the study area was sufficient for *Synechococcus* growth at in situ and low temperature, but
375 the nutrient started to become limited when temperature increased (Fig. 2). Our results
376 indicate that an increase in temperature will exacerbate nutrient limitation for *Synechococcus*
377 growth. This phenomenon could be common as it has also been observed in freshwater
378 diatoms and heterotrophic bacteria (Thomas et al. 2017). In comparison with *Synechococcus*,
379 the ambient nutrient concentration was sufficient for *Prochlorococcus* growth at all
380 temperatures as their growth rate showed no significant difference between the two nutrient
381 scenarios (Fig. 2). The temperature sensitivity of *Prochlorococcus* growth was, therefore, not
382 constrained by the nearly depleted nutrient conditions (Figs. 3, 4). We believe that warming
383 will also increase the nutrient demand for *Prochlorococcus* growth, but its demand is usually

384 extremely low and easily satisfied. *Prochlorococcus* have adapted themselves to the
385 extremely oligotrophic environments by reducing their cell and genome sizes to minimize the
386 resource demands (Partensky and Garczarek 2010). The small cell size leads to a large
387 surface-to-volume ratio, facilitating efficient nutrient acquisition (Raven 1998). The
388 'streamlined genome', which is much smaller than that of *Synechococcus*, allows
389 *Prochlorococcus* to reduce their nutrient requirements and grow solely on the extremely low
390 amount of regenerated NH_4^+ from remineralization processes (Partensky and Garczarek
391 2010). In addition, warming will also increase the supply of regenerated nutrient by
392 accelerating microbial activities especially in the ecosystems featured high nutrient recycling
393 by the microbial loop, which could balance the increase of nutrient demands for
394 phytoplankton growth. Thus, *Prochlorococcus* is not prone to be limited by the current low
395 nutrient concentration even under warming conditions. In contrast, increasing nutrient
396 regeneration cannot satisfy the increasing demands for the growth of *Synechococcus* under
397 warming conditions. The diminishing nutrient supply in the future warmer ocean will,
398 therefore, further limit the growth of *Synechococcus* and curtail its thermal response.
399 Nevertheless, predicting the potential response of *Prochlorococcus* and *Synechococcus* to the
400 ocean warming should further take into account their adaptive behaviours. For instance,
401 phytoplankton can adapt to the temperature changes by re-allocating their cellular C, P, and
402 N pools to the optimal, subsequently adjusting their nutrient demands (Toseland et al. 2013).
403 Such metabolic adaption cannot be revealed by the short-term experiments but needs further
404 investigation (García et al. 2018).

405 ***How temperature sensitivity of Synechococcus growth being constrained by nutrient***
406 ***limitation?***

407 The temperature sensitivity of *Synechococcus* growth rate was constrained by the
408 almost depleted nutrient conditions in the study area, revealed by the lower apparent

409 temperature sensitivity (E) under in situ nutrient conditions than E_a estimated under nutrient
410 replete conditions (Figs. 3, 4). The meta-analysis on the compiled dataset also showed
411 reduced activation energy of *Synechococcus* growth without adding the effect of nutrient to
412 the calculation (Fig. 6). As a result, the response of *Synechococcus* growth to warming could
413 differ among regions. In nutrient-limited regions, such as the subtropical gyres,
414 *Synechococcus* growth is expected to have a slower response to the increasing temperature
415 because limited nutrient constrains their growth. Nevertheless, in mesotrophic and eutrophic
416 waters, such as upwelling and coastal regions, increasing temperature may significantly
417 stimulate the growth of *Synechococcus*.
418 Although growing evidence reveals the constraining effect of limited nutrient on the
419 thermal response of phytoplankton growth (O'Connor et al. 2009; Marañón et al. 2018), the
420 underlying mechanisms remain ambiguous. One mechanism relates to the enzyme kinetics. It
421 has been intensively studied in terrestrial ecology that enzymatic kinetics accounts for how
422 the limited substances constrain the thermal response of soil organic matter decompositions
423 (German et al. 2012). This mechanism can also explain how nutrient limitation affects the
424 temperature sensitivity of phytoplankton metabolic rate (Marañón et al. 2018).
425 When nutrient is limited, the growth of phytoplankton not only depends on μ_{max} but
426 also on K_n, a parameter characterizing the affinity of enzymes for the nutrient substance. It
427 has been found that K_n for phytoplankton nutrient uptake and growth under nitrogen,
428 phosphate, or silicate limitation is also temperature sensitive, and so is μ_{max} (Bestion et al.
429 2018). An increase in K_n (i.e., decrease in the affinity of enzymes and kinetic efficiency) with
430 increasing temperature probably stems from the thermal adaption of the relevant proteins.
431 Increasing temperature will change the structure of proteins which regulate the K_n and
432 catalytic rate constant (K_{cat}) by changing their key amino acid residues, rendering the
433 'flexible' proteins more 'rigid' and less active in ligand binding and recognition (Somero

2004). The less 'flexible' proteins ultimately result in a lower affinity of the enzyme towards substrate as well as a higher K_n. For the enzymatic reaction of soil organic matter decomposition, the temperature sensitivity of K_n will counteract the thermal response of maximum reaction rate, thereby leading to a reduced temperature sensitivity of decomposition in soils (German et al. 2012). Hence, the reduced temperature sensitivity of phytoplankton growth under nutrient limitation could also be attributed to the counteracting effect of the thermal response of μ_{max} and K_n (Marañón et al. 2018). In the present study, the robust K_n value and its temperature sensitivity cannot be calculated due to the extremely low nutrient concentrations in the experiments. Nevertheless, the positive correlation between μ_n/μ_0 ratio and temperature suggested that K_n for *Synechococcus* growth would be temperature-dependent and increasing with temperature (Fig. 5). In addition, when fitting the data of the compiled dataset to the explicit model (*Eq. 5*) involving the temperature effect on K_n, a significant activation energy of K_n was obtained, consistent with the elevated activation energy of *Synechococcus* growth rate under saturated nutrient conditions (Table S1).

However, differing from previous studies that reported a severe suppression of temperature sensitivity of phytoplankton growth and metabolic rates by nutrient limitation (Marañón et al. 2014; 2018), the temperature sensitivity of *Synechococcus* growth was only reduced by 37% due to nutrient limitation in our experiments (Fig. 4). This difference may stem from the opposing effects of the temperature sensitivity of μ_{max} and K_n and beg the questions as to what determines the E and the extent of the reduction in temperature sensitivity.

Based on the explicit model of phytoplankton growth rate, which is a combination of the effects of temperature, nutrient and light, involving the temperature effects on both growth rate and K_n (logarithmic transformation of *Eq. 6*):

458 $$ln\mu_0 = ln\mu_c + \frac{E_a}{k_b}\left(\frac{1}{T_c} - \frac{1}{T}\right) + ln\frac{N}{N+k_0 e^{\frac{E_k}{k_b}(\frac{1}{T_c}-\frac{1}{T})}} + \ln(1 - e^{-\frac{\alpha_I PAR}{\mu_c}}) \qquad (7)$$

459 the apparent temperature sensitivity (E) can be estimated through the derivative of the
460 function with respect to temperature ($d\ln\mu_0/dT$). As the experiments in our study
461 (temperature modulated experiments and compiled dataset) were conducted in the surface
462 layers where light is sufficient for the growth of *Synechococcus* (Fig. 6b), the effect of light
463 on growth rate was temperature-independent. Thus, we treated the last term of *Eq. 7* as
464 constant when calculating $d\ln\mu_0/dT$. However, the light intensity could also affect the
465 temperature sensitivity of phytoplankton growth rate when it becomes a limiting factor
466 (Edwards et al. 2016). To simplify the equation, we let x represents the Boltzmann
467 temperature $\frac{1}{k_b}\left(\frac{1}{T_c} - \frac{1}{T}\right)$. Then:

468 $$E = \frac{dln\mu_0}{dx} = E_a - \frac{k_0 e^{E_k x}}{N+k_0 e^{E_k x}} E_k \qquad (8)$$

469 Assuming $N = n\ K_n$ ($n > 0$), the E equals to $E_a - 1/(n+1)\ E_k$. Based on this inference, E is
470 determined by not only the activation energy of μ_{max} and K_n (E_a and E_k, respectively) but also
471 the level of nutrient limitation (n). Under nutrient replete conditions, the E approximately
472 equals to E_a because the $1/(n+1)\ E_k$ is negligible when n is large enough. While when
473 nutrient concentration is extremely low ($N << K_n$), E will approach E_a - E_k and reach the
474 lowest value, which could be 0 when the E_k is very similar with E_a. Thus, the limited
475 reduction of temperature sensitivity of *Synechococcus* growth in our study may be because
476 the nutrient limitation was not so severe for *Synechococcus* growth in the study region (Fig.
477 2). Besides, the E_k of K_n for *Synechococcus* growth could be much smaller than E_a of
478 *Synechococcus* growth, leading to a weak opposing effect on their thermal response and a
479 relatively small reduction in their temperature sensitivity (Figs. 4, 5).

480 ***Higher E_a estimated in the temperature modulated experiments***

481 The E_a estimates in our study are all higher than the reported E_a for the bulk
482 phytoplankton growth rate (0.3 - 0.4 eV), which is claimed to be lower than heterotrophic
483 processes (Allen et al. 2005; Chen and Laws 2017; Liu et al. 2019). Nevertheless, our results
484 are consistent with recent findings that the prokaryotes have higher temperature sensitivity
485 than eukaryotes (Chen et al. 2014; Chen and Laws 2017; Smith et al. 2019). This difference
486 will significantly affect carbon cycling in the warming and expanding oligotrophic ocean
487 (Smith et al. 2019).
488 The E_a estimated from experiments for *Prochlorococcus* and *Synechococcus* growth
489 (1.02 ± 0.33 eV and 1.80 ± 0.29 eV, respectively, Table 2) were higher than the values
490 estimated from the compiled dataset using GAMs and explicit nonlinear model (0.63 ± 0.07
491 eV and 0.49 ± 0.10 eV, respectively) which were close to the classic values predicted by
492 MTE (0.65 eV, Brown et al. 2004). This could be the difference between the temperature
493 sensitivities derived within and across populations. In the temperature modulated dilution
494 experiments, E_a revealing the emergent response of the same *Prochlorococcus* and
495 *Synechococcus* populations to the increasing temperature during the incubation is the within-
496 populations temperature sensitivity. By contrast, the E_a estimated from the meta-analysis on
497 the complied dataset were the across-population temperature sensitivity as the dataset
498 consisted of the growth rates of various *Prochlorococcus* and *Synechococcus* populations
499 from a variety of environments. Previous studies on E_a estimates were usually derived across
500 species based on the dataset of a variety of phytoplankton species (Eppley 1972; Chen and
501 Laws 2017). Some species and populations can modulate their ability to adapt to the
502 environments and increase their growth rates during adaptation, which partially compensates
503 the emergent thermal response of phytoplankton within populations or species (Chen and
504 Laws 2017; Barton and Yvon-Durocher 2019). Thus, the across-population E_a estimated from
505 meta-analysis was lower than the emergent E_a estimated from the short-term experiments.

506 The high emergent E_a values were consistent with the estimates for many *Prochlorococcus*
507 and *Synechococcus* strains in laboratory experiments (Stawiarski et al. 2016; Chen and Laws
508 2017; Barton and Yvon- Durocher 2019). Moreover, the high E_a could also arise from stress
509 reactions of *Prochlorococcus* and *Synechococcus* to the abrupt temperature changes in such
510 temperature manipulated experiments at short-term scales, which could be alleviated by
511 acclimation and long-term adaptation. Indeed, it is noteworthy that the temperature sensitivity
512 derived from short-term temperature modulated experiments cannot be applied at long-term
513 adaptation scales. Therefore, the E_a estimated based on short-term experiments, such as our
514 study, should be used with great cautions in predicting how marine plankton respond to the
515 projected warming which occurs gradually over a long period of time.
516
517 **Conclusion**
518 We have provided the first field evidence of nutrient-dependent temperature
519 sensitivity of cyanobacterial populations in the subtropical northwest Pacific, one of the
520 warmest regions of the global ocean. Our results suggest that *Prochlorococcus* are well
521 adapted to the current environmental temperature and extremely low nutrient conditions.
522 *Synechococcus* are more plastic in thermal adaptation and their response to the increasing
523 temperature will be constrained by limiting nutrient supply. Thus, the growth of
524 *Prochlorococcus* are less susceptible to nutrient depletion (or availability) but more
525 vulnerable to warming, while the effect of temperature on the growth of *Synechococcus* and
526 other large phytoplankton will be affected by nutrient availability. As such, a significantly
527 different thermal response could be seen in plankton communities between mesotrophic and
528 eutrophic waters (e.g., upwelling, coastal regions), and oligotrophic ocean (e.g., subtropical
529 gyres). We further verified that the response of phytoplankton growth to increasing
530 temperature under nutrient limitation should be determined by the temperature dependence of

enzyme kinetics related to growth (K_n) as well as the levels of nutrient limitation. Our study points to the importance of considering nutrient availability in evaluating how phytoplankton growth and primary production will respond to the projected ocean warming, particularly in the oligotrophic ocean.

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Acknowledgements

The authors sincerely thank the captain, officers, and the crew of the R/V *Hakuho Maru* for their helpful support during the KH-16-7 expedition. We are grateful to three anonymous reviewers for their helpful comments, Drs. J. Nishioka and K. Yoshida for nutrient analyses, Miss S. Jiang for providing some dilution data, and Dr. J. Liu for editing the manuscript. This study was conducted by the "Study of Kuroshio Ecosystem Dynamics for Sustainable Fisheries (SKED; JPMXD0511102330)" by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan and supported by the Hong Kong Branch of Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou; SMSEGL20SC01). HL wishes to acknowledge the support by research grants from the Research Grants Council of Hong Kong (16101917 and 16101318).

The authors declare no conflict of interest.

718 **Figure legends**

719 Fig. 1 Locations of experimental stations in the subtropical northwest Pacific. The map was
720 drawn by Ocean Data View (Schlitzer, 2013. Ocean Data View http://odv.awi.de)

721 Fig. 2 Boxplot of the growth rates of *Prochlorococcus* and *Synechococcus* at 6 stations under
722 different temperature treatments (Chilling, In situ T and Warming). μ_0: in situ growth
723 rate without nutrient addition; μ_n: growth rate with nutrient enrichment. The dots are the
724 outliers of the boxplot. The *p* value and star means the significant levels between μ_0 and
725 μ_n (paired *t*-test: * : $p < 0.5$; ** : $p < 0.1$; *** : $p < 0.001$).

726 Fig. 3 The growth rates of *Synechococcus* (a) and *Prochlorococcus* (b) for each experiment.
727 The solid and dotted lines are the regression lines of linear mixed effects model on the
728 growth rates with and without nutrient enrichment, respectively. Dots: the growth rates
729 with nutrient enrichment. Triangle: the growth rates without nutrient enrichment. E_a:
730 activation energy of growth rate under nutrient-enriched condition. *E*: activation energy
731 of growth rate under ambient nutrient condition. Open dots or triangles: data not used
732 in the linear mixed effects models.

733 Fig. 4 Activation energy of the growth rate of *Prochlorococcus* and *Synechococcus*. The two
734 dashed lines represent the theoretical activation energy of autotrophic processes (0.32
735 eV, Allen et al. 2005) and heterotrophic processes (0.65 eV, Brown et al. 2004). The *p*
736 value and star means the significant levels between apparent activation energy (*E*) and
737 activation energy (E_a) (* : $p < 0.5$; ** : $p < 0.1$; *** : $p < 0.001$).

738 Fig. 5 The ratios of μ_n/μ_0 for *Prochlorococcus* and *Synechococcus* under different
739 experimental temperatures. The dashed line is the OLS regression on the temperature
740 and μ_n/μ_0 of *Synechococcus* (slope = 0.074 with *p* value of 0.015 (<0.05)).

741 Fig. 6 Effects of temperature, photosynthetically active radiation (PAR), and nitrate
742 concentration on the growth rates of *Synechococcus* and *Prochlorococcus* (ln μ_0 or

Relative ln μ_0 (difference of ln μ_0 from the mean)). Solid lines: the smoothing lines estimated from GAMs with shaded areas representing 95% CI; blue dash lines: the ordinary least squares (OLS) regression of growth rate and temperature; E_a: activation energies estimated from GAMs including the effects of light and nutrient; E: apparent activation energy estimated from OLS regression.