

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

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

16 **Running head:** Nutrient limitation reduces *Synechococcus* thermal sensitivity

17

18 **Abstract**

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

37

## 38 **Introduction**

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

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

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

63 2008). Phytoplankton will thereby experience a more nutrient-impoverished situation in the  
64 warming ocean. Under nutrient-limited conditions, as in the oligotrophic ocean,  
65 phytoplankton growth was found to be controlled by nutrient concentrations rather than  
66 temperature (Marañón et al. 2014). The thermal response of phytoplankton growth rate could,  
67 therefore, be constrained and suppressed by the nutrient limitation in nature (O'Connor et al.  
68 2009; Marañón et al. 2018). Nevertheless, the mechanism underpinning the constraining  
69 effect of nutrient availability remains elusive, which hinders a better evaluation and  
70 prediction of how the primary production will respond to the projected ocean warming with  
71 consequent nutrient impoverishment.

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

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

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

111

## 112 **Materials and Methods**

### 113 *Dilution experiments in the subtropical northwest Pacific*

114 *Experimental set-up.* The growth rates of *Synechococcus* and *Prochlorococcus* at  
115 three different temperatures were measured by temperature modulated dilution experiments  
116 conducted aboard the R/V *Hakuho Maru* (JAMSTEC/ University of Tokyo) in December  
117 2016 in the Kuroshio Current and its adjacent waters in the subtropical northwest Pacific  
118 Ocean (Fig. 1). The dilution approach was designed to measure the phytoplankton growth  
119 and microzooplankton grazing rates simultaneously via diluting the natural plankton  
120 communities with in situ particle-free seawater to certain proportions and incubating them for  
121 one day (Landry and Hassett 1982). The rates were calculated based on the linear relationship  
122 of dilution factors and net growth rates by assuming that the growth rate of phytoplankton is  
123 not affected by the dilution treatments and the grazing rate of microzooplankton is  
124 proportional to the dilution factors (the proportion of original seawater). To measure the rates  
125 at different temperatures, the prescribed mixtures of plankton communities were incubated at  
126 designated temperatures: in situ surface temperature (T), T-4°C, and T+4°C. At each station,  
127 we used an acid-washed plastic bucket to collect the surface seawater and then gently  
128 transferred them into two 20-litre polycarbonate carboys.

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

137 to achieve the 25% dilution level. This unfiltered seawater was pre-screened through a 200  
138  $\mu\text{m}$  mesh to remove mesozooplankton. Another two 2.4 L polycarbonate bottles were filled  
139 with pre-screened unfiltered natural seawater to obtain the 100% dilution treatment. Nutrients  
140 ( $\text{NH}_4^+$ : 0.5  $\mu\text{M}$ ,  $\text{PO}_4^{3-}$ : 0.03  $\mu\text{M}$ ,  $\text{Fe}^{3+}$ : 1.0 nM;  $\text{Mn}^{2+}$ : 0.1 nM in final concentrations) were  
141 added to the four bottles to ensure no nutrient limitation. As the phytoplankton communities  
142 were dominated by picocyanobacteria, the nitrogen was added as  $\text{NH}_4^+$  instead of  $\text{NO}_3^-$  which  
143 cannot be utilized by most *Prochlorococcus* strains (Landry et al. 1995; 2011; Moore et al.  
144 2002). To estimate the in situ phytoplankton growth rate, which could be limited by the  
145 ambient nutrient concentrations, two extra bottles filled with pre-screened unfiltered seawater  
146 without adding nutrient were prepared as controls. All bottles were tightly capped and put  
147 into on-deck incubators with designated temperatures for one day. The in situ temperature  
148 was maintained by running surface seawater. The other two temperatures were maintained by  
149 two temperature controllers (EYELA CA-1100 and CTP-3000). Neutral density plastic film  
150 was used to cover all bottles to imitate the in situ light conditions. The carboys, bottles, filters  
151 and silicon tubing used in the experiments were sequentially washed with 10% HCl,  
152 deionized water, Milli-Q water and in situ seawater before each experiment.

153 *Nutrients and phytoplankton analyses.* Samples for determining inorganic nutrients  
154 were taken, frozen at  $-80\text{ }^\circ\text{C}$  immediately, stored in  $-20\text{ }^\circ\text{C}$  freezer, and analysed by a  
155 QuAatro autoanalyzer (BL TEC K.K., Osaka, Japan) with certified reference material  
156 (Aoyama et al., 2012). The detection limits for  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ , and  $\text{NH}_4^+$  are 0.01  $\mu\text{M}$ , 0.05  $\mu\text{M}$ ,  
157 and 0.01  $\mu\text{M}$ , respectively. For Chl *a* analysis, seawater (2.4 L) was filtered onto GF/F filters  
158 (Whatman) under low vacuum, stored in  $-80\text{ }^\circ\text{C}$  freezer, and analysed by ultra-high  
159 performance liquid chromatography (UHPLC) according to the method of Suzuki et al.  
160 (2015).

161 At the beginning and the end of each experiment, samples for flow cytometric (FCM)  
162 analyses were taken to measure the initial and final cell abundances of *Synechococcus* and  
163 *Prochlorococcus*. The samples (2 mL seawater) were fixed with 0.2% buffered  
164 paraformaldehyde (v/v, final concentration), frozen in liquid nitrogen immediately, and stored  
165 in -80 °C freezer until analysis. We used a Becton-Dickson FACSCalibur flow cytometer  
166 equipped with an air-cooled argon laser (488 nm) to enumerated the cell abundances of  
167 *Synechococcus* and *Prochlorococcus*. The *Prochlorococcus* could be detected by the side  
168 scatter (SSC) and red auto-fluorescence emitted by Chl *a* at 680 nm, while the *Synechococcus*  
169 could be distinguished from *Prochlorococcus* because of their particular orange auto-  
170 fluorescence emitted by phycoerythrin at 575 nm (Olson et al. 1993). To normalize and  
171 calibrate the fluorescence and light scattering signals, fluorescent beads (1 µm, Polysciences,  
172 Inc.) were added to every sample as an internal standard. Aliquots (600 µL) were run for 2 or  
173 3 minutes on the flow cytometer at a calibrated flow rate (~56 µL min<sup>-1</sup>) and the raw data  
174 were analysed using WinMDI software 2.9 (Joseph Trotter, Scripps Research Institute, La  
175 Jolla, CA, USA).

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



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

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

$$195 \quad \mu = \mu_c e^{-E_a/k_b T} \quad (1)$$

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

$$208 \quad \ln\mu_{i,j} = (\ln\mu_c + \theta_{ri}) + \frac{E_a + \theta_{E_a i}}{k_b} \left( \frac{1}{T_c} - \frac{1}{T_{i,j}} \right) + \varepsilon_{i,j} \quad (2)$$

209 where  $\mu_{i,j}$  is the growth rate of *Synechococcus* or *Prochlorococcus* at  $j^{\text{th}}$  temperature  $T_{i,j}$  (K)  
 210 at  $i^{\text{th}}$  station ( $\mu_n$  or  $\mu_0$ ),  $\mu_c$  is the normalized rate at reference temperature  $T_c$  (288 K),  $\theta_{ri}$  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  $\mu_n$  should not be  
 213 affected by nutrient availability, whilst the  $E_a$  of  $\mu_0$  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  $\mu_n$  and  $\mu_0$ . 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:

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

224 in which  $\mu$  is the growth rate,  $\mu_{max}$  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  $\mu_n$  and  $\mu_0$ . The  $\mu_n$  estimated under nutrient-enriched conditions in the  
 229 dilution experiments should be equal to  $\mu_{max}$  in Eq. 3, while the instantaneous growth rates  
 230 ( $\mu_0$ ) 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  $\mu_n/\mu_0$   
238 in our study based on the formula above. Thus, instead of calculating  $K_n$ , we used the ratio of  
239  $\mu_n/\mu_0$  to represent  $K_n$  and the intensity of nutrient limitation. In fact,  $\mu_n/\mu_0$  (generally  
240 expressed as  $\mu_0/\mu_n$ ) is widely used as a nutrient limitation index of phytoplankton in dilution  
241 experiments (Landry et al. 1995). The relationship between the  $\mu_n/\mu_0$  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 ( $\mu_0$ ) and nutrient-enriched growth rate ( $\mu_n$ ).  
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<sup>-2</sup> d<sup>-1</sup>) 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 ( $\mu_0$ ) according to Eq.  
256 1:

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

258 where  $\mu_{c1}$  is the normalized rate at reference temperature  $T_c$  (288 K),  $T$  is the temperature (K)  
259 corresponding with  $\mu_0$ . The Ordinary Least Squares (OLS) regression was used for the  
260 calculation and performed with the function “*lm*” in R.

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

$$264 \quad \ln \mu_0 = \ln \mu_{c2} + \frac{E_a}{k_b} \left( \frac{1}{T_c} - \frac{1}{T} \right) + s(PAR) + s(\ln(N)) \quad (5)$$

265 where  $\mu_0$  is instantaneous growth rates used in the Eq. 4,  $\mu_{c2}$  is the normalization constant,  $N$   
 266 is the nitrate concentration,  $PAR$  is the photosynthetically active radiation, other symbols are  
 267 the same with Eq. 2.  $N$  was log-transformed before GAMs analysis to follow the quasi-  
 268 normal distribution. The GAMs analysis was implemented by R function “*gam*” in the  
 269 package “*mgcv*” (Wood 2006).

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

$$273 \quad \mu_0 = \mu_{c3} e^{\frac{E_a}{k_b} \left( \frac{1}{T_c} - \frac{1}{T} \right)} \frac{N}{N + k_0 e^{\frac{E_k}{k_b} \left( \frac{1}{T_c} - \frac{1}{T} \right)}} \left( 1 - e^{-\frac{\alpha_I PAR}{\mu_c}} \right) \quad (6)$$

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

278

## 279 **Results**

280 Affected by the Kuroshio Current, surface waters of the study region were  
 281 characterized by high temperature (~25 °C) and extremely low nutrient with  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$   
 282 concentrations below the detection of quantification (Table 1). Chl  $a$  concentrations were low  
 283 with a range of 0.17–0.32  $\mu\text{g L}^{-1}$ . *Prochlorococcus* abundance averaged 84100 cells  $\text{mL}^{-1}$ ,

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  $1.36 \pm 0.59$  eV and  $1.19$   
300  $\pm 0.20$  eV, respectively (Table 2, Figs. 3, 4). Under the nutrient-enriched condition,  $E_a$  of  
301 *Prochlorococcus* growth ( $1.02 \pm 0.33$  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 ( $E_a = 1.80 \pm 0.29$  eV,  $p = 0.021 < 0.05$ ;  
305 Table 2, Fig. 3, 4).

306 Nearly all the  $\mu_n/\mu_0$  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

309 between  $K_n$  for *Prochlorococcus* and temperature. In contrast, the  $\mu_n/\mu_0$  ratios of  
310 *Synechococcus* were positively correlated with temperature (spearman  $R = 0.52$ ,  $p < 0.05$ ),  
311 which suggested that  $K_n$  of *Synechococcus* growth increased with increasing temperature  
312 (Fig. 5).

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

327

## 328 Discussion

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

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

### 336 ***Thermal responses of Prochlorococcus and Synechococcus growth rate***

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

355 The sea surface temperature in this region is relatively high (~ 25 °C). However,  
356 artificially elevating temperature still stimulates the growth of *Synechococcus* at all  
357 experimental stations (Fig. 3a), which indicates that the optimal temperature for  
358 *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  $\text{NH}_4^+$  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  $\mu_{max}$  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  $\mu_{max}$  (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

434 2004). The less ‘flexible’ proteins ultimately result in a lower affinity of the enzyme towards  
435 substrate as well as a higher  $K_n$ . For the enzymatic reaction of soil organic matter  
436 decomposition, the temperature sensitivity of  $K_n$  will counteract the thermal response of  
437 maximum reaction rate, thereby leading to a reduced temperature sensitivity of  
438 decomposition in soils (German et al. 2012). Hence, the reduced temperature sensitivity of  
439 phytoplankton growth under nutrient limitation could also be attributed to the counteracting  
440 effect of the thermal response of  $\mu_{max}$  and  $K_n$  (Marañón et al. 2018). In the present study, the  
441 robust  $K_n$  value and its temperature sensitivity cannot be calculated due to the extremely low  
442 nutrient concentrations in the experiments. Nevertheless, the positive correlation between  
443  $\mu_n/\mu_0$  ratio and temperature suggested that  $K_n$  for *Synechococcus* growth would be  
444 temperature-dependent and increasing with temperature (Fig. 5). In addition, when fitting the  
445 data of the compiled dataset to the explicit model (Eq. 5) involving the temperature effect on  
446  $K_n$ , a significant activation energy of  $K_n$  was obtained, consistent with the elevated activation  
447 energy of *Synechococcus* growth rate under saturated nutrient conditions (Table S1).

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

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

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

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 
$$E = \frac{d\ln\mu_0}{dx} = E_a - \frac{k_0 e^{E_k x}}{N+k_0 e^{E_k x}} E_k \quad (8)$$

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  $\mu_{max}$  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 ( $N \ll K_n$ ),  $E$  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 ( $1.02 \pm 0.33$  eV and  $1.80 \pm 0.29$  eV, respectively, Table 2) were higher than the values  
490 estimated from the compiled dataset using GAMs and explicit nonlinear model ( $0.63 \pm 0.07$   
491 eV and  $0.49 \pm 0.10$  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 compiled 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

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

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717

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).  $\mu_0$ : in situ growth  
723 rate without nutrient addition;  $\mu_n$ : growth rate with nutrient enrichment. The dots are the  
724 outliers of the boxplot. The  $p$  value and star means the significant levels between  $\mu_0$  and  
725  $\mu_n$  (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  $\mu_n/\mu_0$  for *Prochlorococcus* and *Synechococcus* under different  
739 experimental temperatures. The dashed line is the OLS regression on the temperature  
740 and  $\mu_n/\mu_0$  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* ( $\ln \mu_0$  or



743 Relative  $\ln \mu_0$  (difference of  $\ln \mu_0$  from the mean)). Solid lines: the smoothing lines  
744 estimated from GAMs with shaded areas representing 95% CI; blue dash lines: the  
745 ordinary least squares (OLS) regression of growth rate and temperature;  $E_a$ : activation  
746 energies estimated from GAMs including the effects of light and nutrient;  $E$ : apparent  
747 activation energy estimated from OLS regression.