



Light availability modulates the effects of warming in a marine N₂ fixer

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Received: 25 September 2019 – Discussion started: 30 September 2019

Revised: 23 January 2020 – Accepted: 1 February 2020 – Published: 28 February 2020

Abstract. *Trichodesmium* species, as a group of photosynthetic N₂ fixers (diazotrophs), play an important role in the marine biogeochemical cycles of nitrogen and carbon, especially in oligotrophic waters. How ongoing ocean warming may interact with light availability to affect *Trichodesmium* is not yet clear. We grew *Trichodesmium erythraeum* IMS 101 at three temperature levels of 23, 27, and 31°C under growth-limiting and growth-saturating light levels of 50 and 160 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, respectively, for at least 10 generations and then measured physiological performance, including the specific growth rate, N₂ fixation rate, and photosynthesis. Light availability significantly modulated the growth response of *Trichodesmium* to temperature, with the specific growth rate peaking at $\sim 27^\circ\text{C}$ under the light-saturating conditions, while growth of light-limited cultures was non-responsive across the tested temperatures (23, 27, and 31°C). Short-term thermal responses for N₂ fixation indicated that both high growth temperature and light intensity increased the optimum temperature (T_{opt}) for N₂ fixation and decreased its susceptibility to supra-optimal temperatures (deactivation energy – E_h). Simultaneously, all light-limited cultures with low T_{opt} and high E_h were unable to sustain N₂ fixation during short-term exposure to high temperatures (33–34°C) that are not lethal for the cells grown under light-saturating conditions. Our results imply that *Trichodesmium* spp. growing under low light levels while distributed deep in the euphotic zone or under cloudy weather conditions might be less sensitive to long-term temperature changes that occur on the timescale of multiple generations

but are more susceptible to abrupt (less than one generation time span) temperature changes, such as those induced by cyclones and heat waves.

1 Introduction

In vast areas of the ocean, primary production is usually limited by availability of nitrogen (Moore et al., 2013). In addition to recycling within the euphotic zone, biologically available nitrogen sources can be supplied to phytoplankton from upwelling, aerosol deposition, and N₂ fixation by diazotrophic prokaryotes, supporting new primary production (Dugdale and Goering, 1967). *Trichodesmium* is one of the major diazotrophic organisms occurring in the pelagic oceans (Zehr, 2011). *Trichodesmium* is a genus of filamentous cyanobacteria that exists as both single filaments and colonies consisting of tens to hundreds of trichomes and that is broadly distributed in oligotrophic tropical and subtropical oceans (Capone et al., 1997). Its contribution to local new production can even be more important than that of nitrate diffusion in some regions (Capone et al., 2005; LaRoche and Breitbart, 2005; Mahaffey et al., 2005), and it thus plays a significant role in marine ecosystems and biogeochemical cycles of nitrogen and carbon (Sohm et al., 2011; Zehr, 2011).

Trichodesmium has attracted tremendous research interest since its discovery as a diazotroph in the early 1960s (Dugdale et al., 1961, 1964). Recently, considerable research at-

attention has been focused on evaluating effects of the ongoing ocean climate change, including sea surface warming associated with global warming, on this keystone organism (Fu et al., 2014; Hutchins and Fu, 2017; Jiang et al., 2018; Hutchins et al., 2007). The IPCC RCP8.5 scenario predicts that upper-ocean temperature will increase by about 3°C on average by the end of the 21st century, and the strongest ocean warming will happen in tropical and subtropical regions (Collins et al., 2013). Because of its important role in marine biogeochemical cycles and marine ecosystems, understanding the responses of *Trichodesmium* to ocean warming and their underlying mechanisms will be critical to evaluating the potential implications of climate change on marine primary productivity, food web dynamics, and biogeochemical cycles.

Previous studies demonstrate that without resource limitation the growth-versus-temperature curve is unimodal in *Trichodesmium*, with lower and upper tolerance limits separately at 18–20 and 32–34°C and optimum temperature at 26–28°C (Breitbarth et al., 2007; Chappell and Webb, 2010; Fu et al., 2014). Based on these findings and the spatial heterogeneity of present temperatures and projected warming of *Trichodesmium*'s habitat (Capone et al., 1997; Collins et al., 2013), the effects of ocean warming on *Trichodesmium* can be spatially diverse, generally benefiting those occurring in relatively high latitudes but being harmful to those occurring near to the Equator (Breitbarth et al., 2007; Fu et al., 2014; Thomas et al., 2012). However, this pattern can be distorted and complicated by resource limitations. For example, it is shown that iron limitation, which is commonly experienced by *Trichodesmium* in nature (Hutchins and Boyd, 2016; Sohm et al., 2011), substantially increases the optimum temperature in *Trichodesmium* (Jiang et al., 2018).

Similar to iron availability, light is also among the key environmental drivers for *Trichodesmium* (Breitbarth et al., 2008; Cai and Gao, 2015; Cai et al., 2015). *Trichodesmium* can be distributed from the sea surface down to 150 m depth, where light intensity at noon ranges from >2000 to <10 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (Davis and McGillicuddy, 2006; Olson et al., 2015). Moreover, *Trichodesmium* is known to be able to partially regulate its vertical position in the water column by buoyancy adjustment (Villareal and Carpenter, 2003). Currently, ocean warming effects on *Trichodesmium* have been widely examined under single, light-saturating conditions, providing important knowledge on this diazotroph's physiological responses to temperature changes (Breitbarth et al., 2007; Fu et al., 2014; Jiang et al., 2018; Levitan et al., 2010). However, how it responds to warming under both light-limiting and light-saturating conditions is also of general significance but has been rarely studied (Boatman et al., 2017).

The growth of phytoplankton is a holistic result of many biochemical and physiological activities, so its responses to environmental changes are dependent on species-specific physiology. Normally, warming increases enzyme activities, accelerating biochemical reactions (Gillooly et al., 2001).

For phytoplankton, reduced growth at high temperatures might be the result of less carbon availability due to the higher thermal sensitivity of respiration compared to that of photosynthesis (Padfield et al., 2015). This negative effect of high temperatures might be exacerbated by direct reduction of photosynthesis under light limitation. In the diazotroph *Trichodesmium*, besides photosynthesis and respiration, the N_2 fixation process might also play a critical role in its growth response to environmental changes. Little has been documented on this aspect (Jiang et al., 2018).

In this study, we explored the combined effects of temperature and light in *Trichodesmium erythraeum* IMS 101. We measured the specific growth rate, photosystem functions, and N_2 fixation rate in *Trichodesmium* cultures acclimated to two light levels and three temperatures. Moreover, we measured their short-term thermal responses for N_2 fixation. In this paper, “acclimation” and “acclimated” indicate that cultures were given enough time (several weeks) to respond to the environmental changes so that balanced growth was achieved, and “short term” refers to acute processes and changes occurring within hours. Although adaptation (over several hundreds of generations) has been demonstrated to be critical in evaluating the responses in phytoplankton to environmental changes (Hutchins et al., 2015; Li et al., 2017; Padfield et al., 2015; Schaum et al., 2018; Tong et al., 2018), it is beyond the scope of this study.

2 Materials and methods

2.1 Culture conditions

Triplicate cultures of *Trichodesmium erythraeum* (strain IMS101, originally isolated from the North Atlantic Ocean by Prufert-Bebout et al., 1993) were established under six different culture conditions. These included factorial combinations of three temperatures (23 ± 1 , 27 ± 1 , and $31 \pm 1^\circ\text{C}$) and two light intensities (light saturating, $160 \pm 20 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, and light limiting, $50 \pm 6 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$). These three growth temperatures are representatives of present and future temperatures of *Trichodesmium* habitats (Breitbarth et al., 2007; Fu et al., 2014). The light-limiting and light-saturating levels were established based on a pilot experiment (Fig. S1a in the Supplement) and previous studies (Breitbarth et al., 2008; Cai et al., 2015; Garcia et al., 2011). All the cultures were run semi-continuously by continual dilutions (every 2–4 d) in the artificial seawater medium YBCII without combined nitrogen (Chen et al., 1996) in 1 L glass flasks maintained in plant growth chambers (HP300G-C, Ruihua, China). Light was provided by LED tubes (FSL, China) with a 12 : 12 light : dark cycle. Different levels of light intensity were achieved using neutral density filters. Cultures were continuously bubbled with air (outdoor) so that the cyanobacteria floated as single filaments. The cells were allowed to accli-

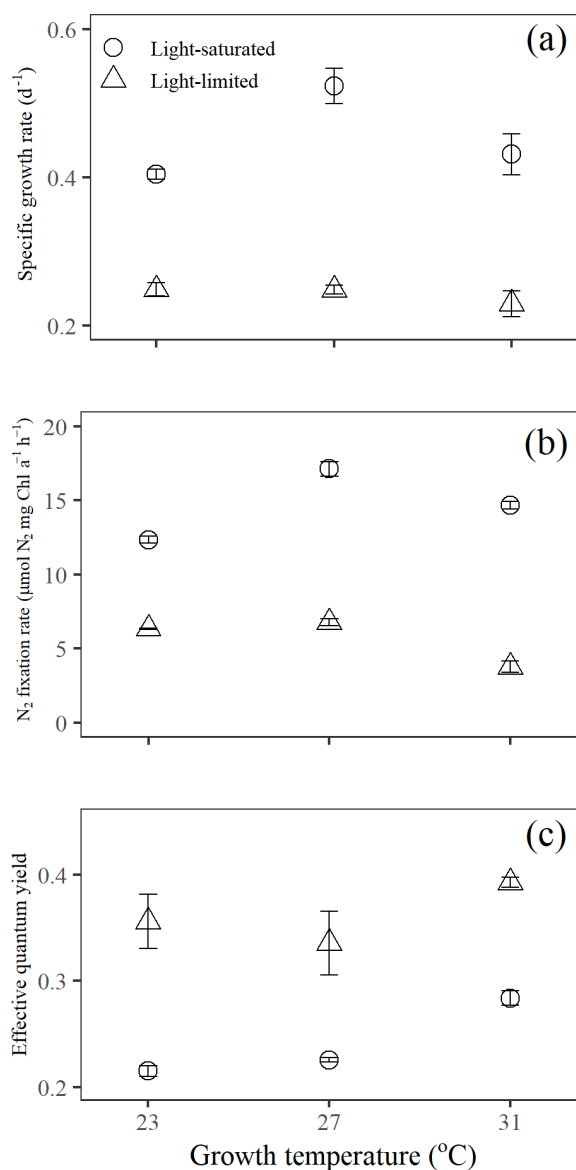


Figure 1. *Trichodesmium* responses of (a) growth, (b) N₂ fixation rate, and (c) effective quantum yield to temperature and light availability interactions. Values represent the mean \pm the standard deviation of biological replicates ($n = 3$).

mate to each condition for at least 10 generations. Acclimation was confirmed by balanced growth with a stable specific growth rate. Then, we started the sampling and data collection.

2.2 Chlorophyll *a* (Chl *a*) concentration and specific growth rate

Chl *a* concentration was spectrophotometrically quantified by gently filtering the cells onto glass-fiber filters (GF/F, Whatman), followed by extraction in pure methanol at 4°C for 24 h and centrifugation at 6000 g for 10 min. The

absorbance spectrum of supernatant was determined using a spectrophotometer (DU800, Beckman, USA). Chl *a* concentration was calculated as [Chl *a*] (microgram per milliliter) = $12.9447 \times (A_{665} - A_{750})$, where A_{665} and A_{750} were, respectively, the absorbances at 665 and 750 nm (Ritchie, 2006).

Because Chl *a* concentration is a good proxy for biomass in *Trichodesmium* (Breitbarth et al., 2007), Chl *a* concentrations measured on different days were analyzed using Eq. (1) to obtain the specific growth rate:

$$\ln(\text{Chl } a(t)) = \mu \cdot t + b, \quad (1)$$

where Chl *a*(t) is the Chl *a* concentration at time t (d), μ is the specific growth rate (d^{-1}), and b is interpreted as the natural logarithm of Chl *a* concentration at day 0. Because the cultures were semi-continuously maintained, Chl *a* concentrations at each time point were corrected by the dilution ratios with the assumption of no dilution.

2.3 Short-term thermal response for N₂ fixation

N₂ fixation rates were determined using the acetylene reduction assay assuming a ratio of 4 : 1 to convert ethylene production to N₂ fixation (Capone, 1993). To examine the responses of N₂ fixation in the cells grown at different temperatures and light levels to short-term temperature changes, we simultaneously measured N₂ fixation at five temperatures, ranging from 19 to 35°C. For each replicate, a 25 mL aliquot of the culture was taken and dispensed into five vials. Five vials, each containing a 5 mL culture, were separately placed in five different zones of two multi-zone culture chambers (HP100–2 and HP100–3, Ruihua, China) and allowed to equilibrate to different target temperatures for 30 min. Pilot experiments had showed that 30 min was enough for temperature equilibrium. After temperature equilibration, each vial was spiked with 1 mL (12.5 % of headspace volume) pure acetylene and incubated for another 30 min under the growth light level. The quantity of ethylene produced was determined using a gas chromatograph with a flame ionization detector (Clarus 580, PerkinElmer, USA).

Typically, the short-term thermal response curves for N₂ fixation were unimodal and negatively skewed, which could be accommodated to a modified version (Padfield et al., 2015; Schaum et al., 2018) of the Sharpe–Schoolfield model (Schoolfield et al., 1981; Sharpe and DeMichele, 1977):

$$N(T) = N(T_c) \times \exp\left(E_a \times \left(\frac{1}{kT_c} - \frac{1}{kT}\right)\right) / \left(1 + \exp\left(E_h \left(\frac{1}{kT_h} - \frac{1}{kT}\right)\right)\right), \quad (2)$$

where $N(T)$ is the N₂ fixation rate per unit of Chl *a* ($\mu\text{mol N}_2 \text{ mg Chl } a^{-1} \text{ h}^{-1}$) at temperature T (kelvin – K), E_a is the activation energy (electron volt – eV) for N₂ fixation, being indicative of the steepness of the slope leading

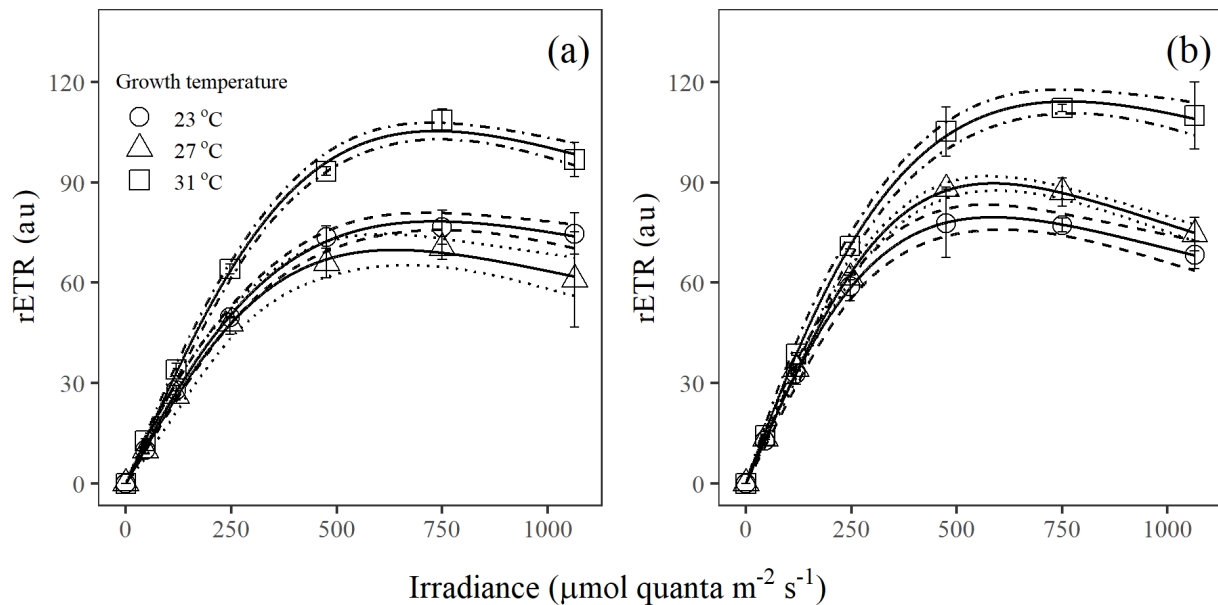


Figure 2. Light response curves of rETR in *Trichodesmium* populations grown under (a) light-saturating and (b) light-limiting conditions (au – arbitrary units). Values represent the mean \pm standard deviation of biological replicates ($n = 3$). Solid lines illustrate the best fit to Eq. (4), with 95 % confidence intervals as dashed lines.

to a thermal optimum, E_h is the deactivation energy (electron volt – eV) characterizing high-temperature-induced inactivation above the deactivation temperature T_h (K), k is Boltzmann’s constant (8.62×10^{-5} eV K $^{-1}$), and $N(T_c)$ is the N_2 fixation rate at an arbitrary reference temperature T_c (here, $T_c = 25^\circ\text{C}$) used for normalization. According to Eq. (2), the optimum temperature (T_{opt} K) corresponding to the maximal N_2 fixation rate (N_{max} , $\mu\text{mol } N_2 \text{ mg Chl } a^{-1} \text{ h}^{-1}$) is

$$T_{\text{opt}} = E_h \times T_h / (E_h + k \times T_h \cdot \ln(E_h/E_a)). \quad (3)$$

Additionally, we also obtained the N_2 fixation rate at the growth temperature (N_{growth}) by inserting corresponding growth temperatures into Eq. (2).

2.4 Chl *a* fluorometry

Photosystem II (PSII) effective quantum yield (Φ_{PSII}) and the photosynthetic relative electron transport rate (rETR) were measured by using the Multiple Excitation Wavelength Chlorophyll Fluorescence Analyzer (MULTI-COLOR-PAM, Walz, Germany) equipped with the US-T temperature control unit (Walz, Germany). Aliquots of 1.5 mL of the culture were taken to determine effective quantum yield (Φ_{PSII}) under actinic light levels that were the same as those of the growth conditions. Then, Φ_{PSII} values were successively measured at seven levels of light intensity (E), ranging from 0 to 1064 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$. Samples were allowed to acclimate to each light level for 3 min before Φ_{PSII} measurements were taken (Suggett et al., 2007). The relative electron transport rate (rETR) at each light level was calculated as $\text{rETR} = E \times \Phi_{\text{PSII}}$ (Ralph and Gademann, 2005). The light

response curve of rETR was analyzed according to the model of Eilers and Peeters (1988):

$$\text{rETR} = \frac{E}{a \cdot E^2 + b \cdot E + c}. \quad (4)$$

Photosynthetic parameters, including the photosynthetic light-harvesting efficiency (α), rETR maximum (rETR_{max}), and light saturation point (E_k), can be calculated as

$$\alpha = \frac{1}{c}, \quad (5)$$

$$\text{rETR}_{\text{max}} = \frac{1}{b + 2 \cdot \sqrt{a \cdot c}}, \quad (6)$$

$$E_k = \frac{c}{b + 2 \cdot \sqrt{a \cdot c}}. \quad (7)$$

During the measurements, the sample temperature was maintained at the corresponding growth temperatures using the US-T temperature control unit (Walz, Germany).

2.5 Statistical analyses

Statistical analyses were performed with the R language (version 3.5.3). [Chl *a*] versus time in each of the triplicate cultures for each treatment was fitted to Eq. (1) using function “lm” in the package “stats” to get the specific growth rate (μ). The significance of differences in specific growth rate (μ) between treatments was tested using the two-way analysis of variance (ANOVA; function “aov” in the package “stats”), followed by Tukey’s test for pairwise comparison (function “TukeyHSD” in the package “stats”). The homo-

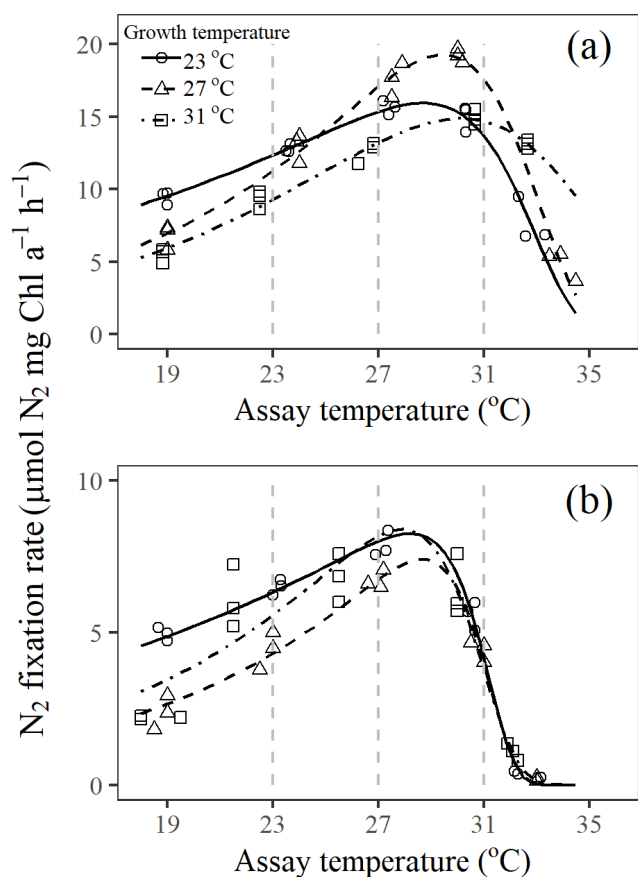


Figure 3. Short-term thermal response curves for N_2 fixation rate in *Trichodesmium* cultures grown under (a) light-saturating and (b) light-limiting conditions. Fitted lines are based on fixed-effect coefficients of the nonlinear mixed-effects model fit to Eq. (2). Vertical dotted lines mark the assay temperatures 23, 27, and 31°C.

generosity of variance assumption and the residual normality assumption were separately checked by Levene's test (function "leveneTest" in the package "stats") and the Shapiro–Wilk test (function "shapiro.test" in the package "stats"). The significance level was set to 0.05.

To test whether parameters $N(T_c)$, E_a , E_h , T_h , and T_{opt} differ between different treatments, we fitted short-term thermal responses for N_2 fixation to Eq. (2) using nonlinear mixed-effects models ("nlme" package). Models included random effects on each of the parameters of Eq. (2) by replicate cultures. The structure of the fixed effects of the nonlinear mixed effects model was determined by trying all possible models (625 models) and selecting the one with the lowest small sample-size corrected Akaike information criterion (AICc; see Supplement Table S3 for all tested models' AICc). The AICc was calculated using function "AICc" in the package "MuMIn".

The light curve of rETR in each of the triplicate cultures for each treatment was fitted to Eq. (4) using function "nls" in the package "stats". The significance of differences in the

photosynthetic light-harvesting efficiency (α), rETR maximum (rETR_{max}), light saturation point (E_k), and effective quantum yield (Φ_{PSII}) between treatments was tested using the same statistical methods as those for μ . The significance level was set to 0.05.

3 Results

3.1 Specific growth rate and N_2 fixation rate

Specific growth rates of *Trichodesmium* IMS 101 were significantly affected by growth light intensity (two-way ANOVA, $F_{1,12} = 662.7$, $P < 0.001$), growth temperature (two-way ANOVA, $F_{2,12} = 22.0$, $P < 0.001$), and the interaction between these two drivers (two-way ANOVA, $F_{2,12} = 18.0$, $P < 0.001$; Table 1). High growth light intensity increased specific growth rates of *Trichodesmium* IMS 101 by 63 % at 23°C (Tukey's test comparing light-saturated and light-limited growth rates at 23°C, $P < 0.001$), 111 % at 27°C (Tukey's test comparing light-saturated and light-limited growth rates at 27°C, $P < 0.001$), and 88 % at 31°C (Tukey's test comparing light-saturated and light-limited growth rates at 31°C, $P < 0.001$; Fig. 1a). The interaction between growth light intensity and temperature in the specific growth rate was indicated by the totally different temperature effects between light-saturated and light-limited cultures. Light-saturated growth rates of *Trichodesmium* IMS 101 were maximal at 27°C, with a value of $0.52 \pm 0.02 \text{ d}^{-1}$ (\pm SD), being higher by 29.5 % (Tukey's test comparing growth rates between light-saturated cultures grown at 27 and 23°C, $P < 0.001$) and 21.3 % (Tukey's test comparing growth rates between light-saturated cultures grown at 27 and 31°C, $P < 0.001$) than those at 23 and 31°C, respectively. However, light-limited growth rates ranged from $0.23 \pm 0.02 \text{ d}^{-1}$ (\pm SD) to $0.25 \pm 0.01 \text{ d}^{-1}$ (\pm SD), with no significant differences between the tested temperatures (Tukey's test comparing growth rates among light-limited cultures grown at three temperatures, $P > 0.05$ for all three comparisons).

Overall, N_2 fixation rates at the growth temperature (N_{growth} ; Fig. 1b) were significantly higher in cultures grown under high light intensity compared to those grown under low light intensity (two-way ANOVA, $F_{1,12} = 149.9$, $P < 0.001$). Also, the growth temperature significantly affected N_{growth} (two-way ANOVA, $F_{1,12} = 3912.3$, $P < 0.001$). Different thermal effects between light-saturated and light-limited cultures indicated a significant interaction between light and temperature in N_{growth} (two-way ANOVA, $F_{2,12} = 112.7$, $P < 0.001$). For light-saturated cultures, the N_{growth} peaked at 27°C, with a value of $17.1 \pm 0.5 \mu\text{mol } N_2 \text{ mg Chl } a^{-1} \text{ h}^{-1}$ (\pm SD), which was higher by 39 % and 17 % than those at 23°C (Tukey's test comparing N_{growth} between light-saturated cultures grown at 27 and 23°C, $P < 0.001$) and 31°C (Tukey's test com-

Table 1. Results of two-way ANOVA for specific growth rate, N₂ fixation rate, effective quantum yield, and parameters derived from rETR light curve with interactions between temperature and light. See Table S2 for results of pairwise comparisons.

Parameter	Effect	degree of freedom (d.f.)	<i>F</i> value	<i>P</i> value
Specific growth rate	Temperature	2,12	22.0	<0.001
	Light	1,12	662.7	<0.001
	Temperature × light	2,12	18.0	<0.001
N ₂ fixation rate (N _{growth})	Temperature	2,12	3912.3	<0.001
	Light	1,12	149.9	<0.001
	Temperature × light	2,12	112.7	<0.001
Effective quantum yield	Temperature	2,12	22.1	<0.001
	Light	1,12	233.2	<0.001
	Temperature × light	2,12	1.8	0.211
rETR light curve: α	Temperature	2,12	24.5	<0.001
	Light	1,12	10.6	<0.01
	Temperature × light	2,12	0.2	0.815
rETR light curve: E_k	Temperature	2,12	5.0	<0.05
	Light	1,12	6.0	<0.05
	Temperature × light	2,12	1.0	0.394
rETR light curve: rETR _{max}	Temperature	2,12	31.2	<0.001
	Light	1,12	139.8	<0.001
	Temperature × light	2,12	8.1	<0.01

paring N_{growth} between light-saturated cultures grown at 27 and 23°C, $P < 0.001$), respectively. However, for light-limited cultures, the value of N_{growth} at 27°C ($6.8 \pm 0.2 \mu\text{mol N}_2 \text{ mg Chl } a^{-1} \text{ h}^{-1}$, $\pm\text{SD}$) was similar to that at 23°C ($6.3 \pm 0.1 \mu\text{mol N}_2 \text{ mg Chl } a^{-1} \text{ h}^{-1}$, $\pm\text{SD}$; Tukey's test comparing N_{growth} between light-limited cultures grown at 27 and 23°C, $P = 0.54$) but significantly higher than that at 31°C ($3.8 \pm 0.4 \mu\text{mol N}_2 \text{ mg Chl } a^{-1} \text{ h}^{-1}$, $\pm\text{SD}$; Tukey's test comparing N_{growth} between light-limited cultures grown at 27 and 31°C, $P < 0.001$).

3.2 PSII effective quantum yield (Φ_{PSII}) and rETR light response curves

Compared to light-saturated cells, light-limited cells had higher values of Φ_{PSII} (Fig. 1c; two-way ANOVA, $F_{1,12} = 233.2$, $P < 0.001$). Meanwhile, under both light regimes, Φ_{PSII} in *Trichodesmium* cultures grown at 31°C was significantly higher than that in cultures grown at 23 and 27°C (two-way ANOVA, $F_{2,12} = 22.1$, $P < 0.001$). No interaction between growth light intensity and temperature in Φ_{PSII} was found (two-way ANOVA, $F_{2,12} = 1.8$, $P = 0.211$).

The rETR light response curve of *Trichodesmium* IMS 101 was influenced by growth temperature in both light-saturated (Fig. 2a) and light-limited (Fig. 2b) treatments. This thermal impact was mainly reflected in the rETR_{max} (two-way ANOVA, $F_{2,12} = 31.2$, $P < 0.001$), which tended to be higher in cultures acclimated to 31°C than those in cultures acclimated to 23°C or 27°C (Table 2; Fig. 2). Addi-

tionally, high growth light intensity tended to decrease the rETR_{max} (two-way ANOVA, $F_{1,12} = 31.2$, $P < 0.001$), especially for cultures grown at 27°C (Tukey's test comparing rETR_{max} between light-saturated and light-limited cultures grown at 27°C, $P < 0.001$). Both high light intensity (two-way ANOVA, $F_{1,12} = 6.0$, $P < 0.05$) and high temperatures (two-way ANOVA, $F_{2,12} = 5.0$, $P < 0.05$) significantly increased the E_k (Table 2).

3.3 Short-term thermal response for N₂ fixation

Optimum temperature (T_{opt}) for N₂ fixation in *Trichodesmium* IMS 101 was affected by both growth temperature and light intensity (Table 3). Generally, T_{opt} for N₂ fixation in light-saturated cultures was higher than that in light-limited cultures, whereas warming effects on N₂ fixation T_{opt} differed between light-saturated and light-limited cultures. For light-saturated cultures, elevations of the growth temperature raised the T_{opt} for N₂ fixation. A 4°C warming was accompanied by a 0.5–0.8°C increase in T_{opt} , which was $28.7 \pm 0.2^\circ\text{C}$ ($\pm\text{SEM}$, standard error of the mean), $29.5 \pm 0.2^\circ\text{C}$ ($\pm\text{SEM}$), and $30.0 \pm 0.3^\circ\text{C}$ ($\pm\text{SEM}$) for the cells grown at 23, 27, and 31°C, respectively. Under light-limiting levels, T_{opt} in cultures grown at 27°C ($T_{\text{opt}} = 28.6 \pm 0.2^\circ\text{C}$, $\pm\text{SEM}$) was higher than that in cultures grown at 23°C ($T_{\text{opt}} = 28.2 \pm 0.2^\circ\text{C}$, $\pm\text{SEM}$), but T_{opt} in cultures grown at 31°C ($T_{\text{opt}} = 27.8 \pm 0.2^\circ\text{C}$, $\pm\text{SEM}$) was the lowest among all treatments. As expected, the maximal N₂ fixation rate (N_{max}) in light-saturated cultures was higher than

Table 2. The light-harvesting efficiency (α), relative electron transport rate maximum (rETR_{max}), and light saturation point (E_k), derived from the light curves (Fig. 2), for *Trichodesmium* grown at different temperature and light intensity levels. Values represent the mean \pm standard deviation of biological replicates ($n = 3$). Superscripts with different letters represent significant difference (Turkey's test; more details in Table S2; $p < 0.05$) among the treatments. The units of E_k and rETR_{max} are micromoles of quanta per square meter per second and arbitrary unit, respectively.

	Growth conditions					
	Light-saturating			Light-limiting		
	23°C	27°C	31°C	23°C	27°C	31°C
α	0.25 \pm 0.01 ^{ac}	0.24 \pm 0.03 ^a	0.28 \pm 0.01 ^c	0.30 \pm 0.03 ^{bc}	0.28 \pm 0.03 ^b	0.35 \pm 0.03 ^b
E_k	316 \pm 22 ^{ab}	322 \pm 45 ^{ab}	371 \pm 16 ^a	270 \pm 17 ^b	319 \pm 38 ^{ab}	329 \pm 21 ^{ab}
rETR _{max}	78 \pm 3 ^a	72 \pm 3 ^a	105 \pm 2 ^b	80 \pm 6 ^a	90 \pm 2 ^c	115 \pm 4 ^b

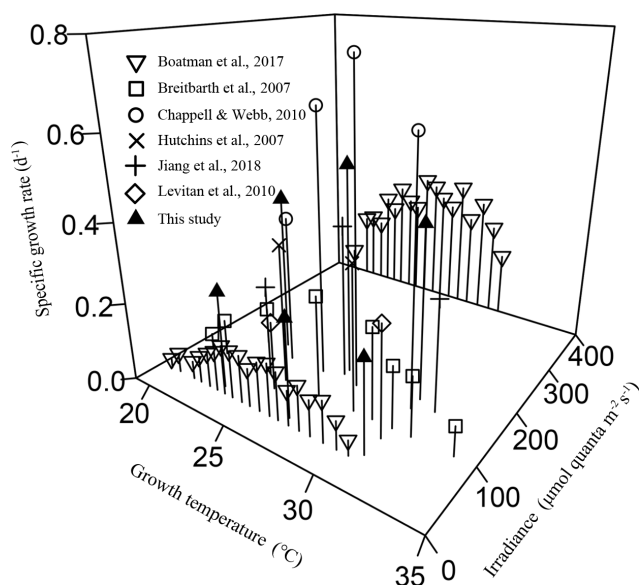


Figure 4. The combined effects of temperature and light intensity on the specific growth rate in *Trichodesmium* IMS 101, with data from the literature involving at least two growth temperatures and this study.

that in light-limited cultures (Table 3). The temperature effect on N_{\max} was also dependent on the light availability. Light-saturated N_{\max} was highest in cultures grown at 27°C ($N_{\max} = 19.3 \pm 0.4 \mu\text{mol N}_2 \text{ mg Chl } a^{-1} \text{ h}^{-1}$, \pm SEM), being higher by 21% and 32% than those grown at 23°C ($N_{\max} = 16.0 \pm 0.3 \mu\text{mol N}_2 \text{ mg Chl } a^{-1} \text{ h}^{-1}$, \pm SEM) and 31°C ($N_{\max} = 14.6 \pm 0.4 \mu\text{mol N}_2 \text{ mg Chl } a^{-1} \text{ h}^{-1}$, \pm SEM), respectively. However, N_{\max} for light-limited cultures was similar among different temperature treatments (Table 3).

The value of deactivation energy (E_h) for N_2 fixation, reflecting the thermal susceptibility to supra-optimal temperatures, was affected by both light availability and growth temperature but not by their interaction (Table 3). E_h tended to be lower in *Trichodesmium* cultures grown under high temperatures and high light intensity. With the highest T_{opt}

($30.0 \pm 0.3^\circ\text{C}$, \pm SEM) and the lowest E_h ($1.47 \pm 0.14 \text{ eV}$, \pm SEM) among all treatments, light-saturated cultures acclimated to 31°C were the only cultures that could maintain considerable N_2 fixation rates at assay temperatures as high as 34°C (Fig. 3). In addition, both light availability and growth temperature affected the deactivation temperature (T_h) for N_2 fixation in *Trichodesmium*, and no interaction between these two drivers was found on T_h (Table 3). T_h in light-saturated cultures tended to be higher than that in light-limited cultures regardless of the growth temperature. T_h was lower in cultures grown at 31°C compared to that in cultures grown at 23 or 27°C under both light levels. The activation energy (E_a) for N_2 fixation was affected by growth temperature but not by growth light intensity (Table 3). The values of E_a for N_2 fixation increased from $0.49 \pm 0.04 \text{ eV}$ (\pm SEM) to $0.91 \pm 0.05 \text{ eV}$ (\pm SEM) and $1.07 \pm 0.04 \text{ eV}$ (\pm SEM) as growth temperatures increased from 23 to 27 and 31°C.

4 Discussion

In this study, light availability not only affected growth rate and N_2 fixation directly but also modulated their responses to temperature changes in *Trichodesmium* IMS 101. Reduced energy supply due to light limitation leads to lowered nitrogen fixation and thus reduced growth in the diazotroph. The specific growth rate was maximal at 27°C for cells grown under saturating light but was virtually insensitive to temperature changes across the tested temperature (23, 27, and 31°C) for light-limited cultures.

The interactions between temperature and light on *Trichodesmium* demonstrated in this work are relevant to natural light and temperature variations and to *Trichodesmium* global change physiology and biogeography. Light supplies energy for photosynthesis, growth, and other key activities, such as N_2 fixation in cyanobacterial diazotrophs. The observed phenomenon that the growth rate becomes less sensitive to temperature changes (Figs. 1a and 4) in *Trichodesmium* IMS 101 under light-limiting levels can be attributed to insufficient energy supply for the cells to respond to temperature changes.

Table 3. Model parameters of thermal responses for N_2 fixation. The structure of the model's fixed effect is $N(T_C) \sim \text{temperature} \times \text{light}$. $E_a \sim \text{temperature}$. $E_h \sim \text{light} + \text{temperature}$. $T_h \sim \text{light} + \text{temperature}$ in which “+” and “*” represent additive and interactive effects, respectively.

Parameter	Light	Temperature (°C)	Estimate	SEM	CI (95 %)
$N(T_C)$ ($\mu\text{mol N}_2 \text{ mg Chl } a^{-1} \text{ h}^{-1}$)	Light-saturated	23	38.3	1.0	[36.4, 40.3]
		27	39.1	1.0	[37.1, 41.1]
		31	41.3	1.5	[38.2, 44.3]
	Light-limited	23	19.5	0.8	[18.0, 21.1]
		27	15.0	0.8	[13.4, 16.6]
		31	20.3	1.1	[18.2, 22.5]
E_a (eV)	No light effect	23	0.49	0.04	[0.41, 0.57]
		27	0.91	0.05	[0.80, 1.01]
		31	1.07	0.04	[0.98, 1.16]
E_h (eV)	Light-saturated	23	4.49	0.51	[3.47, 5.51]
		27	3.99	0.31	[3.36, 4.61]
		31	1.47	0.14	[1.18, 1.75]
	Light-limited	23	7.51	0.68	[6.15, 8.87]
		27	7.01	0.60	[5.82, 8.21]
		31	4.49	0.49	[3.50, 5.48]
T_h (°C)	Light-saturated	23	32.6	0.1	[32.3, 32.9]
		27	32.4	0.2	[32.1, 32.8]
		31	31.8	0.2	[31.3, 32.3]
	Light-limited	23	31.1	0.1	[30.9, 31.4]
		27	31.0	0.1	[30.7, 31.2]
		31	30.3	0.2	[29.9, 30.6]
T_{opt} (°C)	Light-saturated	23	28.7	0.2	[28.2, 29.1]
		27	29.5	0.2	[29.2, 29.8]
		31	30.0	0.3	[29.5, 30.6]
	Light-limited	23	28.2	0.2	[27.9, 28.6]
		27	28.6	0.2	[28.3, 28.9]
		31	27.8	0.2	[27.4, 28.2]
N_{max} ($\mu\text{mol N}_2 \text{ mg Chl } a^{-1} \text{ h}^{-1}$)	Light-saturated	23	16.0	0.3	[15.3, 16.6]
		27	19.3	0.4	[18.5, 20.1]
		31	14.6	0.4	[13.9, 15.4]
	Light-limited	23	8.3	0.3	[7.6, 8.9]
		27	7.4	0.4	[6.6, 8.2]
		31	8.6	0.4	[7.8, 9.4]

While thermal biological responses are mainly based on enzymatic performance, light limitation suppresses syntheses of enzymes (Raven and Geider, 1988) and thus subsequently limits thermal responses. Although light-limited phytoplankton cells typically allocate more resources to light-harvesting systems to compensate for light shortages, at very low irradiances this compensation cannot prevent light-harvesting capacity from being a limiting factor for enzyme synthesis and growth (Raven and Geider, 1988). Field investigations show that vertical distributions of *Trichodesmium* can reach depths greater than 100 m, where light is absolutely limiting and temperature is lower compared to surface tempera-

ture (Olson et al., 2015; Rouco et al., 2016). According to the typical values of surface solar irradiances and the vertical extinction coefficient in tropical and subtropical oceans (Olson et al., 2015), the daily light dose received by the light-limited cultures in our study corresponds to that received by *Trichodesmium* at a depth of 50–60 m. The contribution of biomass and N_2 fixation by *Trichodesmium* at depths greater than 50 m might range from 7 % to 28 % (Davis and McGillicuddy, 2006; Olson et al., 2015). Therefore, the evaluation of potential warming effects on *Trichodesmium* should not be constrained to the populations inhabiting light-saturated environments (upper tens of meters; Breitbarth et

al., 2007; Jiang et al., 2018), making 3-D models indispensable. In existing 3-D model studies involving *Trichodesmium* (Boyd and Doney, 2002; Moore et al., 2002), the combined effects of temperature and light on *Trichodesmium* biological activities are simply assumed to be additive, which is proven to be inappropriate in this work. While the absolute values of the N₂ fixation rate under light-limiting and light-saturating levels cannot be directly compared on the basis of Chl *a* content, since lower light levels resulted in more cellular Chl *a* content (Fig. S1b), comparison of the thermal response patterns can generate useful information for improving model predictions of diazotrophic responses to ocean climate change.

Thermal responses for organisms are known to be useful in evaluating thermal acclimation potential and probing low and high temperature tolerances (Gundersen et al., 2010; Somero, 2010; Way and Yamori, 2014). In this work, the shape of the short-term thermal response curves for N₂ fixation is normalization-independent because cells were exposed to different assay temperatures for only 1 h, hardly changing the elemental stoichiometry or cellular pigment component. When exposed to abrupt temperature gradients, the light-saturated cells acclimated to higher temperatures exhibited higher T_{opt} values (Table 3) and lower thermal susceptibility to supra-optimal temperatures (E_{h} ; Table 3). This indicates an increased capability for the diazotroph to tolerate short-term warming impacts. However, light limitation made the cells more susceptible to warming due to decreased T_{opt} and increased E_{h} for N₂ fixation (Table 3). Moreover, with light limitation, acclimation to high temperatures did not help *Trichodesmium* cells tolerate short-term supra-optimal temperatures. On the other hand, Chl *a* fluorescence data show that the PSII in light-limited cultures was as healthy as that in cells grown under saturating light (Fig. 1c, 2), and it has been shown that damage to PSII usually occurs at temperatures above 45°C (Yamori et al., 2014). Therefore, the collapse of N₂ fixation at high temperatures was not likely caused by the dysfunction of the photosystems but might be caused by the uncoupling of adenosine triphosphate (ATP) synthesis to electron transport, since proton leakiness of the thylakoid membrane has been frequently proposed as a problem at high temperatures (Yamori et al., 2014). This is consistent with the observation that supra-optimal temperature inhibition of N₂ fixation was aggravated by light limitation (Fig. 3). In addition, damage to nitrogenase at high temperatures might also be one of the reasons responsible for the faster drop of N₂ fixation at high temperatures in light-limited cultures (Gallon et al., 1993). This is because the extra investment of resources to repair damaged nitrogenase could not be supported under light-limiting conditions (Fig. 3b). Therefore, light availability exerts critical control on the acclimation potential of N₂ fixation in *Trichodesmium* to warming.

Acclimation to different temperatures also affected the activation energy (E_{a}) for N₂ fixation in *Trichodesmium* IMS 101 (Table 3). For *Trichodesmium* species, N₂ fixation

can be controlled by supply of ATP and reducing equivalents, mainly coming from photosynthesis, and the inherent catalytic capacity of the nitrogenase. These two processes may exhibit different temperature dependence, i.e., different E_{a} . The E_{a} of the controlling process determines the N₂ fixation E_{a} (Hikosaka et al., 2006; Staal et al., 2003). Therefore, the differences in N₂ fixation E_{a} between cultures grown at different temperatures may reflect that N₂ fixation was primarily controlled by different processes in cultures acclimated to different temperatures. Preliminary evidence supporting this hypothesis came from the various effects of assay light intensity on the values of E_{a} for N₂ fixation between light-limited cultures grown at 23 and 27°C (Table S1; Fig. S2). For *Trichodesmium* grown under light-limiting levels, the lower E_{a} values in cultures acclimated to 23°C were significantly elevated by the increased assay light intensity which can provide more ATP and reducing equivalents (Table S1; Fig. S2a). This suggests that the constraint should be the supply of ATP and reducing equivalents. The higher E_{a} values in cultures acclimated to 27°C were insensitive to the assay light intensity changes, suggesting that N₂ fixation should not be controlled by the supply of ATP and reducing equivalents at this optimal temperature but may possibly be controlled by inherent catalytic capacity of the nitrogenase (Table S1; Fig. S2b).

The short-term thermal responses for N₂ fixation mirror thermal shock responses. If cells are exposed to the thermal changes for longer time, acclimation will definitely change the thermal responses for N₂ fixation in *Trichodesmium* (Breitbarth et al., 2007; Fu et al., 2014; Staal et al., 2003). To compare the short-term and acclimated thermal responses for N₂ fixation, we calculated the corresponding values of E_{a} , E_{h} , and T_{opt} , which are, respectively, 0.93 ± 0.64 eV (\pm SEM), 1.86 ± 1.19 eV (\pm SEM), and 27.1 ± 1.0 °C (\pm SEM) for fully acclimated N₂ fixation within the range of 20–34°C growth temperatures in *Trichodesmium* IMS 101 (Breitbarth et al., 2007). These values of E_{a} and E_{h} are comparable to those derived from short-term thermal response for N₂ fixation in the same strain grown under light-saturating conditions and 31°C in our study (Table 3), but the T_{opt} value is lower than that from short-term thermal response. On the other hand, we tried to derive values of E_{a} , E_{h} , and T_{opt} for acclimated N₂ fixation rates in another three *Trichodesmium erythraeum* strains (strains RLI, KO4-20, and 21-75; Fu et al., 2014), but the model fitting failed to converge. Instead of being negatively skewed, the thermal response curves of acclimated N₂ fixation in these three *Trichodesmium* strains are nearly symmetrical. These comparisons show that thermal response for N₂ fixation in *Trichodesmium* is strain-specific and is affected on the timescale of acclimation process.

In the oceans, *Trichodesmium* and other pelagic phytoplankton are often exposed to abrupt temperature changes due to strongly disturbed weather conditions, such as tropical cyclones and marine heat waves. Global warming has been predicted to increase both tropical cyclone intensities and the frequency of the most intense tropical cyclones (El-

ner et al., 2008; Knutson et al., 2010; Wehner et al., 2018). Upper-ocean temperature declines prior to and during a cyclone event and then increases abruptly afterwards (Li et al., 2009), accompanied by strong variations in surface solar radiation and stratification (Sriver and Huber, 2007). These abrupt temperature changes occurring in nature are not as acute as those in our experiment. For example, temperature changes caused by cyclones and heat waves are on the order of 0.5–1°C per day (Babin et al., 2004; Beca-Carretero et al., 2018). Nonetheless, these temperature changes occur within one generation of *Trichodesmium* because of its low growth rate, not leaving enough time for full acclimation. Therefore, the values of E_a , E_h , T_h , and T_{opt} provided in this study can likely serve as proxies for some types of abrupt natural temperature increases.

Data availability. The data are available upon request to the corresponding author (Kunshan Gao).

Supplement. The supplement related to this article is available online at: <https://doi.org/10.5194/bg-17-1169-2020-supplement>.

Author contributions. KG and XY designed the experiment. XY carried out the experiment. XY, FXF, DAH, and KG analyzed the data and wrote the paper.

Competing interests. The authors declare that they have no conflict of interest.

Financial support. This research has been supported by the National Key R&D Program of China (grant no. 2016YFA0601400), the National Natural Science Foundation of China (grant nos. 41720104005 and 41721005), the Joint Project of the National Natural Science Foundation of China and Shandong Province (grant no. U1606404), and the US National Science Foundation (grant nos. OCE 1538525, OCE 1657757, and OCE 1638804).

Review statement. This paper was edited by Carol Robinson and reviewed by three anonymous referees.

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