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Light availability modulates the effects of warming in a marine N_2 fixer

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Abstract. As a group of photosynthetic N_2 fixers (diazotrophs), *Trichodesmium* species play an especially 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 ℃ under two growth limiting and saturating light levels of 50 and 160 μmol quanta m^{-2} s^{-1}, respectively, for at least 10 generations, and then measured physiological performances. Light availability significantly modulated the growth response of *Trichodesmium* to temperature, with the specific growth rate peaking at ~27 ℃ under the light–saturating conditions, while growth of light–limited cultures was non–responsive across the temperature range of 23–31 ℃. When the acclimation of N_2 fixation to growth temperatures was evaluated by short–term temperature norms, the optimum temperature (T_{opt}) for N_2 fixation increased by 0.6–1.4 ℃ in the cells grown under high levels of temperature and light, and the susceptibility to supra–optimal temperatures (deactivation energy, E_h) was decreased by 56%–61%. However, light limitation decreased the T_{opt} by 0.5–1.8 ℃ and increased the supra–optimal temperature susceptibility by 33%–71%. This made all light–limited cultures unable to sustain N_2 fixation during short–term exposure to higher temperatures (33–34 ℃) that are not lethal for cultures 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 more susceptible to ocean warming.

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1. Introduction

In vast areas of the oceans, 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 N_2 fixation by diazotrophic prokaryotes, supporting new primary production (Dugdale and Goering, 1967). Obviously, biological N_2 fixation is an important component of the marine biological CO_2 pump (Sohm et al., 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). Among the diazotrophs occurring in the pelagic oceans, *Trichodesmium* is the most well studied group (Bergman et al., 2013; Capone et al., 1997; Zehr, 2011), and has long been recognized as one of the major diazotrophic organisms in the open oceans (Martínez–Pérez et al., 2016; Zehr, 2011). 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 Breitbarth, 2005; Mahaffey et al., 2005), and it thus plays a significant role in global biogeochemical cycles of nitrogen and carbon (Sohm et al., 2011; Zehr, 2011).

Trichodesmium has attracted tremendous research interest for about a century, especially since its discovery as diazotroph in the 1960s (Bergman et al., 2013; Capone et al., 1997). Recently, considerable research attention has been focused on evaluating effects of the ongoing ocean climate changes, including sea surface warming associated with global warming, on this keystone organism (Fu et al., 2014; Hutchins and Fu, 2017; Jiang et al., 2018). In the IPCC RCP 8.5 scenario, upper ocean temperature will increase by about 3 ℃ 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). Understanding the responses of *Trichodesmium* to ocean warming and their underlying mechanisms will be critical to evaluating the potential consequences of climate changes.

Field observations demonstrate that *Trichodesmium* occurrence is generally restricted to waters with sea surface temperatures (SST) between 20 ℃ and 30 ℃ (Breitbarth et al., 2007; Capone et al., 1997). *Trichodesmium's* lower limit is set by the physiological constraint of thermal tolerance, whereas the upper limit is set by the present SST maximum (Breitbarth et al., 2007). Laboratory studies show that the upper limit of *Trichodesmium* thermal tolerance is approximately 32–34 ℃ (Breitbarth et al., 2007; Fu et al., 2014). The growth rate versus temperature curve of *Trichodesmium* is unimodal,

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with an optimum temperature at ~27 ℃ (Breitbarth et al., 2007; Fu et al., 2014; Jiang et al., 2018). Because of the spatial heterogeneity of present temperatures and projected warming of *Trichodesmium's* habitat (Capone et al., 1997; Collins et al., 2013), the responses of *Trichodesmium* to warming have been suggested to be spatially diverse (Breitbarth et al., 2007; Fu et al., 2014; Jiang et al., 2018; Thomas et al., 2012).

The response of growth to environmental change is a holistic result of many biochemical and physiological activities, which can differ from short acclimation times to long adaptation periods. This reflects an organism's acclimation and adaptive strategies to deal with environmental change (Somero, 2010). Thermal acclimation potentials of photosynthesis and respiration can be key growth responses to temperature changes in phytoplankton (Padfield et al., 2015). In the diazotroph *Trichodesmium*, besides photosynthesis and respiration, N_2 fixation process might also play a critical role in its growth response to environmental changes, such as warming. Nevertheless, little has been documented on this aspect (Jiang et al., 2018).

Light is well known to modulate the responses of photosynthetic organisms to environmental change, and light levels and photoperiodicity are particularly important in regulating metabolic activities related to N_2 fixation capacity in *Trichodesmium* (Breitbarth et al., 2008; Cai and Gao, 2015). *Trichodesmium* spp. can be distributed from the sea surface down to 120 m depth (Davis and McGillicuddy, 2006; Olson et al., 2015), where light intensity could be as low as 2 μmol quanta m^{-2} s^{-1}. Ocean warming effects on *Trichodesmium* have been widely examined under single, constant light 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 saturating conditions is also of general significance, considering its dynamic vertical distribution. In the present work, we investigated how temperature and light interactively affect *Trichodesmium*'s N_2 fixation and growth, and found that the cells acclimated to different temperatures and light levels exhibited differential physiological performances in terms of growth, photosynthesis and N_2 fixation, and differential thermal acclimation potential of N_2 fixation.

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2. Material and methods

2.1 Culture conditions

Three replicate populations 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±1 ℃) and two light intensities (saturating light, 160 ± 20 and limiting light, 50 ± 6 μmol quanta m^{-2} 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 limiting and saturating light levels were established based on a pilot experiment (Supplementary Fig. S1(a)). All the cultures were run semi–continuously by continual dilutions (every 2–4 days) 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 cyanobacterial filaments were floating singly. The cells were allowed to acclimate to each condition for at least 10 generations before 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 ℃ for 24 h and centrifugation at 6000g for 10 min. The absorbance spectrum of the supernatant was determined from 400 nm to 700 nm using a spectrophotometer (DU800, Beckman, USA). Chl–a concentration was calculated as: [Chl–a] (μg mL^{-1}) =12.9447*(A_{665}–A_{750}), where A_{665} and A_{750} were respectively the absorbances at 665 and 750 nm (Ritchie, 2006). Specific growth rate was calculated as the slope of the linear regression of the natural log of Chl–a versus time during consecutive dilutions (Hong et al., 2017). Because the cultures were semi–continuously maintained, Chl–a concentrations at each time point was obtained by taking dilution ratios into account before natural logarithm.

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2.3 Short–term temperature norm of N_2 fixation

N_2 fixation rates were determined using the acetylene reduction assay assuming a ratio of 4:1 to convert ethylene production to N_2 fixation (Capone, 1993). To examine the responses of N_2 fixation in the cells grown at different temperatures and light levels to acute temperature changes, we simultaneously measured N_2 fixation at five temperatures ranging from 19 to 35 ℃. For each population, a 25ml aliquot of the culture was taken and dispensed into five vials. Five vials 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 0.5 h. Pilot experiments had showed that 0.5 h 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 0.5 h under the growth light level. The quantity of ethylene produced was determined using a gas chromatograph with flame ionization detector (Clarus 580, PerkinElmer, USA).

Typically, the acute thermal response curves for N_2 fixation were unimodal and negatively skewed, which could be accommodated to a modified Sharpe–Schoolfield model (Padfield et al., 2015; Schoolfield et al., 1981):

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

where N(T) is the N_2 fixation rate (μmol N_2 mg Chl–a^{-1} h^{-1}) at temperature T (Kelvin, K), E_a is the activation energy (electron volt, eV) for N_2 fixation, E_h is the deactivation energy (electron volt, eV) characterizing high temperature induced inactivation above deactivation temperature T_h (K), and $N(T_c)$ is the N_2 fixation rate at the reference temperature T_c (25 ℃) used for normalization. E_a, E_h, T_h and $N(T_c)$ are the parameters obtained through non–linear least squares regression using the 'nlsLM' function in the 'minpack.lm' package, and optimum temperature (T_{opt}) for N_2 fixation was calculated using 'optimize' function in R language. Bringing the T_{opt} into Eq. (1) gives the maximal N_2 fixation rate (N_{max}). Similarly, we can also get the N_2 fixation rate corresponding to the respective growth temperature (N_g). Unexpectedly, the thermal response curves of N_2 fixation in light–limited populations grown at 31 ℃ cannot be described by the Eq. (1) (more details in Sect. 3.3). Therefore, for this treatment, we use N_2 fixation rate measured at 30 ℃ (the assay temperature closest to 31℃) as its N_g.

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2.4 Chl–a fluorometry

Photosystem II (PSII) effective quantum yield (Φ_{PSII}) and photosynthetic relative electron transport rate (rETR) were measured by using the Multiple Excitation Wavelength Chlorophyll Fluorescence Analyzer (MULTI–COLOR–PAM, Walz, German) equipped with the US–T temperature control unit (Walz, Germany). Aliquots of 1.5 m 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 μmol quanta m^{-2} s^{-1}. Samples were allowed to acclimate to each light level for 3 min before Φ_{PSII} measurements (Suggett et a., 2007). Relative electron transport rate (rETR) at each light level was calculated as: rETR = E * Φ_{PSII} (Ralph and Gademann, 2005). The light response curve of rETR was analysed according to the model of (Eilers and Peeters, 1988):

$$rETR = \frac{E}{a*E^2+b*E+c} \quad (2)$$

where a, b and c are parameters that can be obtained through non–linear least squares regression in R language.

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

$$\alpha = \frac{1}{c} \quad (3)$$

$$rETR_{max} = \frac{1}{b+2*\sqrt{a*c}} \quad (4)$$

$$E_k = \frac{c}{b+2*\sqrt{a*c}} \quad (5)$$

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

3. Results

3.1 Specific growth rate and N_2 fixation rate

Specific growth rates of *Trichodesmium* IMS 101 in light–saturated cultures were higher than those in light–limited cultures by 63% at 23 ℃, 111% at 27 ℃ and 88% at 31 ℃, respectively ($P<0.05$, Tukey's HSD method; Fig. 1(a)). Within the temperature range of 23–31 ℃, light–saturated growth rates of *Trichodesmium* IMS 101 were maximal at 27 ℃ with a mean

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value of 0.52 d^{-1}, 29.5% and 21.3% higher than those at 23 ℃ and 31 ℃, respectively ($P<0.05$, Tukey's HSD method). However, light–limited growth rates ranged from 0.23 – 0.25 d^{-1}, without showing any maximal value at any temperature levels tested and with no significant differences across the different temperatures ($P>0.05$, Tukey's HSD method), although the mean growth rate at 31℃ was slightly lower than that at 23 ℃ or 27 ℃.

N_2 fixation rates at growth temperature (N_g) were higher under the growth–saturating light than under the limiting level by 94% at 23 ℃, 149% at 27 ℃ and 128% at 31 ℃ ($P<0.05$, Tukey's HSD method; Fig. 1(b)). Light availability also modulated the effects of growth temperature on N_g (Fig. 1(b)). As with specific growth rate, the N_g peaked at 27 ℃ under light–saturating conditions, but N_g was insensitive to growth temperature change under the light–limiting conditions ($P>0.05$, Tukey's HSD method).

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} ($P<0.05$, Tukey's HSD method; Fig. 1(c)). Meanwhile, under both light regimes, Φ_{PSII} in *Trichodesmium* populations grown at 31 ℃ was significantly higher than that in cultures grown at 23 ℃ and 27 ℃ ($P<0.05$, Tukey's HSD method; Fig. 1(c)). The rETR light response curve of *Trichodesmium* IMS 101 was influenced by growth temperature in both light–saturated (Fig. 2(a)) and light–limited (Fig. 2(b)) treatments. This thermal impact was mainly reflected in the $rETR_{max}$, which tended to be higher in populations acclimated to 31 ℃ ($P<0.05$, Tukey's HSD method; Table 1).

3.3 Short–term temperature norm of N_2 fixation

Generally, the N_2 fixation rates exposed to acute temperature changes were well–described by the modified Sharpe–Schoolfield model (Eq. (1)) in *Trichodesmium* IMS 101 grown under the saturating (Fig. 3(a)) and limiting (Fig. 3(b)) light levels. The only exception is the light-limited cultures grown at 31 ℃ whose N_2 fixation rates showed nearly insensitivity to temperature changes within an assay temperature range of 23–30 ℃, which made the model fitting unsuccessful (Fig. 3(b), Fig. 4).

Under saturating light level, the maximal N_2 fixation rate (N_{max}) corresponding to the optimum temperature (T_{opt}) was highest in cultures grown at 27 ℃, with a mean value of 19.3 μmol N_2 mg Chl–a $^{-1}$ h^{-1}, 21% and 30% higher than those

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grown at 23 ℃ and 31 ℃, respectively (Fig. 4(a); P<0.05, Tukey's HSD method). T_{opt} for N_2 fixation in light–saturated cultures was slightly but significantly increased by growth temperature increase (Fig. 4(b)). An 8 ℃ warming (from 23 ℃ to 31 ℃) was accompanied by 1.4 ℃ increase of T_{opt} (from 28.7 ℃ to 30.1 ℃) (p<0.05, Tukey's HSD method). Simultaneously, the values of deactivation energy (E_h), reflecting the thermal susceptibility to supra–optimal temperatures, in cultures acclimated to 31℃ were 61% and 56% lower than those in cultures acclimated to 23 ℃ and 27 ℃, respectively (Fig. 4(c)). Higher T_{opt} and lower E_h in light–saturated cultures acclimated to 31℃ made them the only treatment that could maintain considerable N_2 fixation rates at assay temperatures as high as 34 ℃ (Fig. 3(a)). Light limitation decreased the N_{max} by 7.9 and 12.1 μmol N_2 mg Chl–a^{-1} h^{-1}, decreased T_{opt} by 0.5 and 1.1 ℃, and increased E_h by 3.3 and 1.4 eV at 23 ℃ and 27 ℃, respectively (Fig. 4(a–c); Table S1). There is no thermally driven difference in N_2 fixation T_{opt}, N_{max} and E_h in light–limited cultures (p>0.05, Tukey's HSD method). Although we cannot derive the N_2 fixation T_{opt}, N_{max} and E_h in the light–limited cultures grown at 31 ℃, it is evident that acclimation to 31 ℃ did not help light–limited cultures maintain N_2 fixation rates during the short–term exposure to supraoptimal temperatures (Fig. 3(b)). None of the light–limited cultures can sustain N_2 fixation rate at an assay temperature of 34 ℃ (Fig. 3(b)). Unlike N_{max}, T_{opt} and E_h, the activation energy (E_a), representing the thermal dependence of metabolic activity within the range of temperature below the deactivation temperature (T_h) for N_2 fixation, was not affected by light availability, but was increased by higher growth temperature regardless of the light levels (Fig. 4(d)). The mean values of E_a for N_2 fixation increased from 0.50 eV to 1.07 eV as growth temperatures increased from 23 ℃ to 31 ℃ (p<0.05, Tukey's HSD method) in light–saturated cultures. These values in light–limited cultures increased from 0.43 eV at 23 ℃ to 1.01 eV at 27 ℃. Surprisingly, the T_h, above which temperature increase induces negative effects on N_2 fixation, was affected by neither growth temperature nor light availability (p>0.05, Two–way ANOVA; Supplementary TableS1).

4 Discussion

In this study, light availability not only affected growth rate and N_2 fixation directly, but also modulated their responses to temperature change in *Trichodesmium* IMS 101. The specific growth rate and N_2 fixation rate were maximal at 27 ℃ for saturating light–grown cells, but were virtually insensitive to temperature changes within the thermal range of 23–31 ℃ for

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light–limited growth cultures. It appears that reduced energy supply due to light limitation leads to lowered nitrogen fixation and thus negative growth effects on the diazotroph.

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 change (Fig. 5(a)) in *Trichodesmium* IMS 101 under limiting light level can be attributed to energy supply being insufficient for the cells to respond to temperature changes. 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 to depths greater than 100 m, where light is absolutely limiting and temperature is lower (Olson et al., 2015; Rouco et al., 2016). According to the typical values of surface light dose and 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 be >28% and 7%–20%, respectively (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–Dimensional models indispensable. In existing 3–Dimensional model studies involving *Trichodesmium* (Boyd and Doney, 2002; J. K. Moore et al., 2001), the effects of temperature and light on *Trichodesmium* biological activities are simply assumed to be additive, which is proven to be inappropriate in this work. Although the absolute values of N_2 fixation rate under light limiting and saturating levels cannot be directly compared on the basis of Chl–a content, since lower light level resulted in more cellular Chl–a content (Supplementary Fig. S1(b)), comparison of the thermal response patterns generates some useful information that can be used to improve model predictions of diazotrophic responses to ocean climate changes.

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Temperature norms or thermal windows for organisms are known to be useful in evaluating thermal acclimation potential and probing low and high temperature tolerances (Gunderson et al, 2010; Somero, 2010; Way and Yamori, 2014). In this work, the shape of the short–term temperature norm of N_2 fixation is normalization–independent because cells were exposed to different assay temperatures for only one hour, hardly changing the elemental stoichiometry or cellular component. When exposed to abrupt temperature gradients, the cells acclimated to higher temperature and light levels exhibited higher T_{opt} values (Fig. 4(b)) and lower thermal susceptibility to supra–optimal temperatures ($E_{h;}$ Fig. 4(c)). This indicates an increased capability for the diazotroph to tolerate warming impacts. However, this is only true under light–saturating conditions, and light limitation would make the cells more susceptible to warming due to decreased T_{opt} and increased E_h for N_2 fixation (Fig. 3(b)). On the other hand, Chl–a fluorescence data shown that the PSII of light–limited cultures were as healthy as those of cells grown under saturating light (Fig. 1(c), 2), and it has been shown that damage to PSII usually occurs at temperatures above 45 ℃ (Yamori et al., 2014). Therefore, the collapse of N_2 fixation at high temperature 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. This is because proton leakiness of the thylakoid membrane has been frequently proposed as a problem at high temperature (Yamori et al., 2014). This is consistent with the observation that supra–optimal temperature inhibition of N_2 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 N_2 fixation at high temperature in light–limited cultures. This is because the extra investment of resources in repair of damaged nitrogenase could not be supported under light–limiting conditions (Fig. 3(b)). Therefore, light availability exerts critical control on the acclimation potential of N_2 fixation in *Trichodesmium* to warming.

Acclimation to different temperatures also affected the activation energy (E_a) for N_2 fixation in *Trichodesmium* IMS 101 (Fig. 4(d)). For *Trichodesmium* species, N_2 fixation can be controlled by supply of ATP/reducing equivalent, mainly coming from photosynthesis, and the inherent catalytic capacity of the nitrogenase. Both of these may have different temperature dependence, i.e. different E_a. The E_a of the controlling process determines the N_2 fixation E_a (Hikosaka, et al,, 2006; Staal et al., 2003). Therefore, the differences in N_2 fixation E_a between cultures grown at different temperatures may reflect that N_2 fixation was primarily controlled by different processes in cultures acclimated to different temperatures. Preliminary

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evidence supporting this came from the various effects of assay light intensity on the values of E_a for N_2 fixation between light–limited cultures grown at 23 ℃ and 27 ℃ (Supplementary Table S2, Fig. S2). For *Trichodesmium* grown under limiting light level, the lower E_a values in populations acclimated to 23 ℃ was significantly elevated by the increased assay light intensity, which can provide more ATP/reducing equivalents (Supplementary Table S2; Fig. S2(a)). This suggests the constraint is the supply of ATP/reducing equivalents. The higher E_a values in populations acclimated to 27 ℃ were insensitive to the assay light intensity change, suggesting N_2 fixation is not controlled by the supply of ATP/reducing equivalents at this optimal temperature, but may possibly be controlled by inherent catalytic capacity of the nitrogenase (Supplementary Table S2; Fig. S2(b)).

The short–term temperature norms of N_2 fixation mirror thermal shock responses. If cells are allowed to exposed to the thermal changes for longer time, acclimation will definitely change the temperature norms of N_2 fixation in *Trichodesmium* (Breitbarth et al., 2007; Fu et al., 2014; Staal et al., 2003). To compare the short–term and acclimated temperature norms, we calculated the corresponding values of E_a (Fig. 5(b)), E_h (Fig. 5(c)) and T_{opt} (Fig. 5(d)), being respectively 0.93eV, 1.86 eV and 27.1 ℃, for fully–acclimated N_2 fixation within the range of 20–34 ℃ growth temperatures in *Trichodesmium* IMS 101, as reported by Breitbarth et al. (2007). These values of E_a and E_h are comparable to those derived from short–term temperature norms of N_2 fixation rate in the same strain grown under light–saturating condition and 31 ℃ in our study (Fig. 5(b–d)), but the T_{opt} values are lower than those from short–term temperature norms. Additionally, the values of E_a, E_h and T_{opt} for acclimated N_2 fixation rates in another three *Trichodesmium* strains were respectively estimated to be 2.76–4.06 eV, 0.54–0.94 eV and 26.1 ℃ (Fu et al., 2014), being a bit different from the values mentioned above. These comparisons show that temperature norms of N_2 fixation in *Trichodesmium* are strains–specific, and/or are affected by the time scale of acclimation process.

In the oceans, *Trichodesmium* and other pelagic phytoplankton are often exposed to acute temperature changes due to strongly disturbed weather conditions, such as tropical cyclones. Global warming has been predicted to increase both tropical cyclone intensities, and the frequency of the most intense tropical cyclones (Elsner et al. 2008; Knutson et al., 2010; Wehner et al., 2018). Upper ocean temperature declines prior and during cyclone events, and then increases abruptly afterwards (Li et al., 2009), accompanied by strong variations of surface solar radiation and stratification (Sriver and Huber, 2007). The E_a, E_h

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and T_{opt} values for N_2 fixation of *Trichodesmium* IMS 101 obtained in this work for the cells acclimated to different temperatures and light levels can, to some extent, be useful in understanding its responses to stochastic and abrupt temperature changes.

Code/Data availability

All data obtained in this study are in Supplement.

Author contribution

KG and XY designed the experiment. XY carried out the experiment. XY, FXF, DH and KG analysed the data and wrote the manuscript.

Competing interests

The authors declare no competing of interest.

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Table 1 The light harvesting efficiency (α), relative election transport rate maximum ($rETR_{max}$) and light saturation point (E_k), derived from the rapid light curves (Fig. 2), for *Trichodesmium* grown at different temperature and light intensity levels; values represent the means and error bars for the standard deviations of biological replicates (n=3); superscripts with different letters represent significant difference ($p<0.05$) among the treatments. The units of E_k and $rETR_{max}$ are μmol quanta m^{-2} s^{-1} and arbitrary unit, respectively.

	Acclimation					
	HL			LL		
	23 °C	27 °C	31 °C	23 °C	27 °C	31 °C
α	0.25 ± 0.01^{ac}	0.24 ± 0.03^{a}	0.28 ± 0.01^{c}	0.30 ± 0.03^{bc}	0.28 ± 0.03^{b}	0.35 ± 0.03^{b}
E_k	316 ± 22^{ab}	322 ± 45^{ab}	371 ± 16^{a}	270± 17^{b}	319 ± 38^{ab}	329 ± 21^{ab}
$rETR_{max}$	78 ± 3^{a}	72 ± 3^{a}	105 ± 2^{b}	80 ± 6^{a}	90 ± 2^{c}	115 ± 4^{b}

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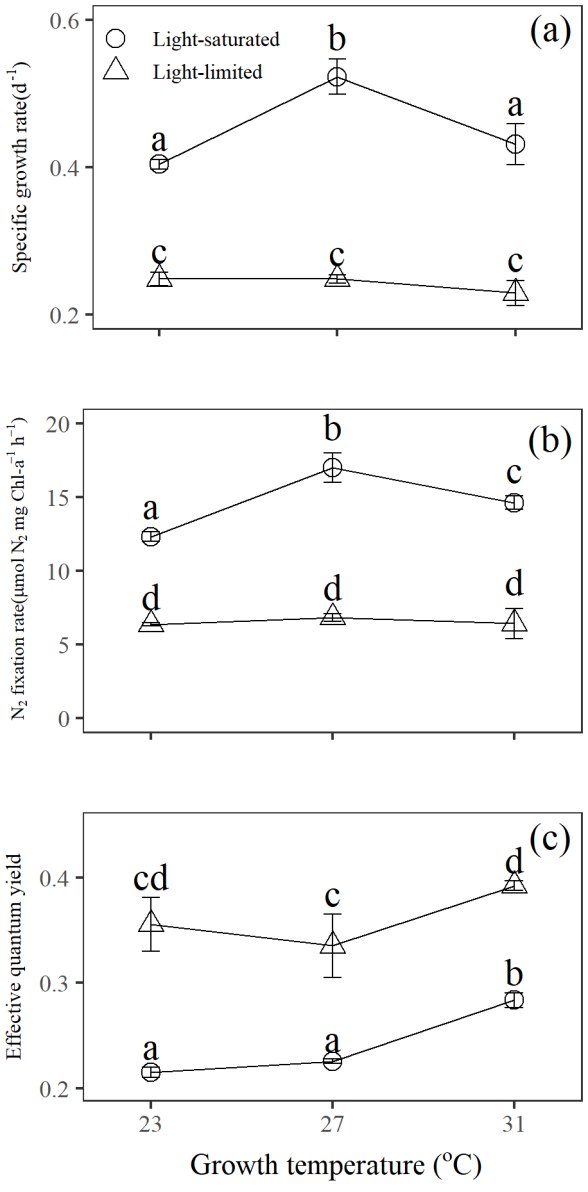

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Figure 1 *Trichodesmium* responses of (a) growth, (b) N_2 fixation rate and (c) effective quantum yield to temperature and light availability interactions; values represent the means and error bars for the standard deviations of biological replicates(n=3); points marked with different letters are significantly different from each other ($p<0.05$).

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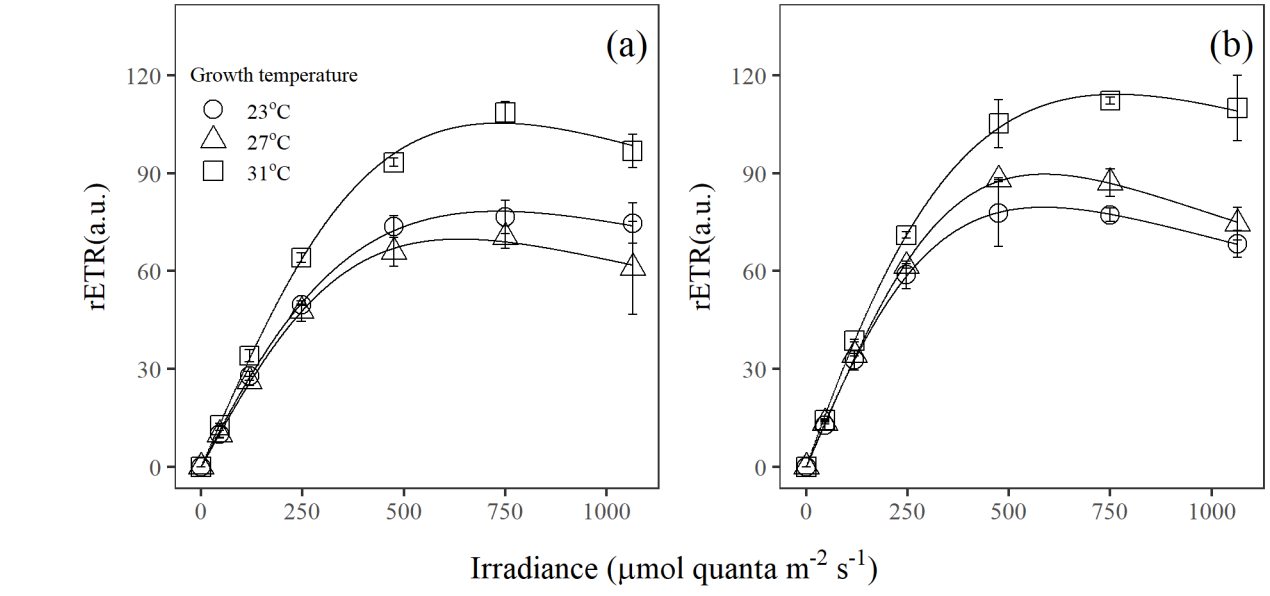

Figure 2 Light response curves of rETR in *Trichodesmium* populations grown under (a) light–saturating and (b) light–limiting conditions; values represent the means and error bars for the standard deviations of biological replicates(n=3); fitted lines are based on mean parameters at each treatment across replicates (n=3) derived from non–linear least squares regression using the Eilers–Peeters model (Eq. (2)).

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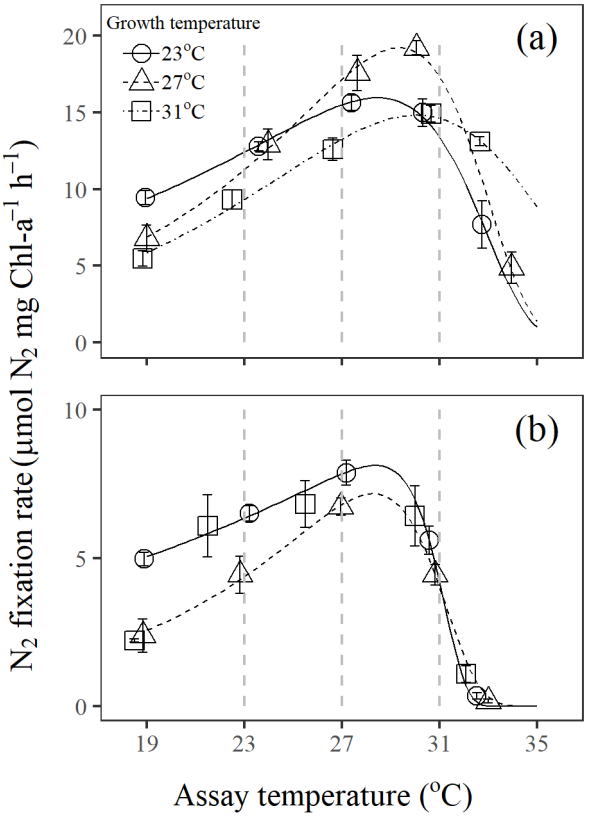

Figure 3 Short–term temperature norms of N_2 fixation rate in *Trichodesmium* populations grown under (a) light–saturating and (b) light–limiting conditions; values represent the means and error bars for the standard deviations of biological replicates(n=3); fitted lines are based on mean parameters at each treatment across replicates (n=3) derived from non–linear least squares regression using the modified Sharpe–Schoolfield model (Eq. (1)); vertical dotted lines mark the assay temperatures 23 °C, 27 °C and 31 °C.

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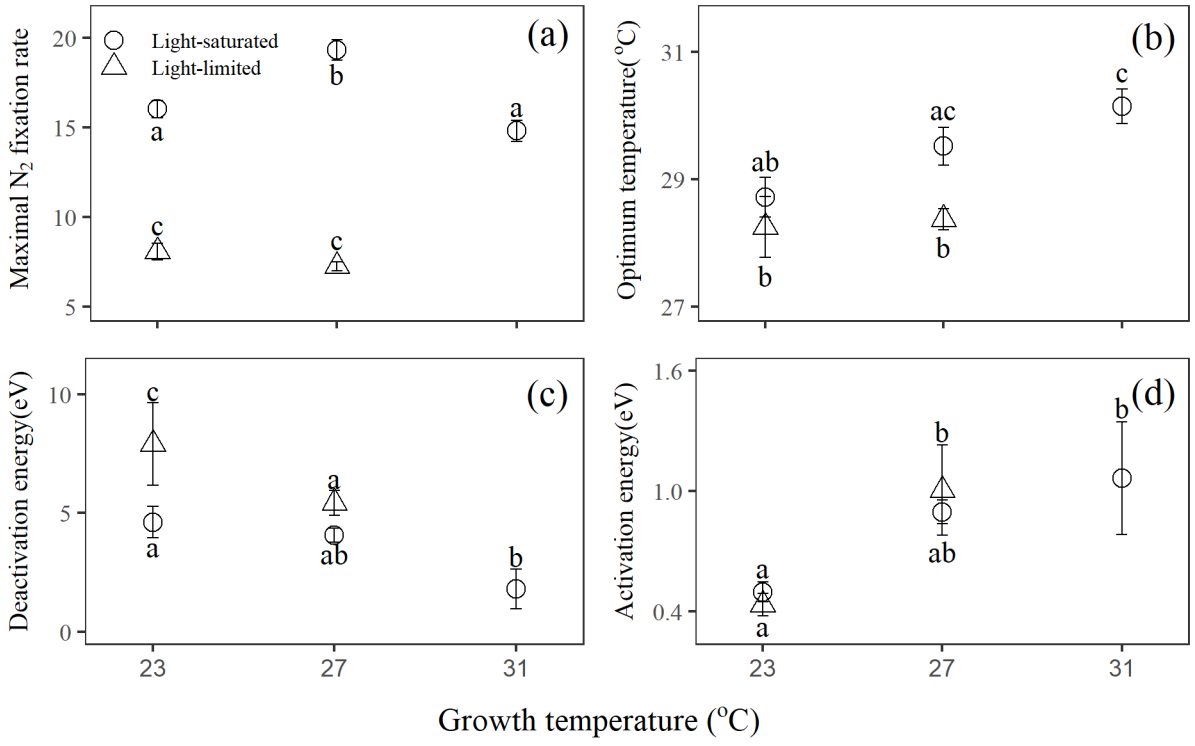

Figure 4 The interactions of temperature and light on (a) maximal N_2 fixation rate, (b) optimum temperature, (c) deactivation energy and (d) activation energy for N_2 fixation in *Trichodesmium*; values represent the means and error bars for the standard deviations of biological replicates(n=3); points marked with different letters are significantly different from each other ($p<0.05$); the unit for maximal N_2 fixation rate is µmol N_2 mg Chl–a^{-1} h^{-1}.

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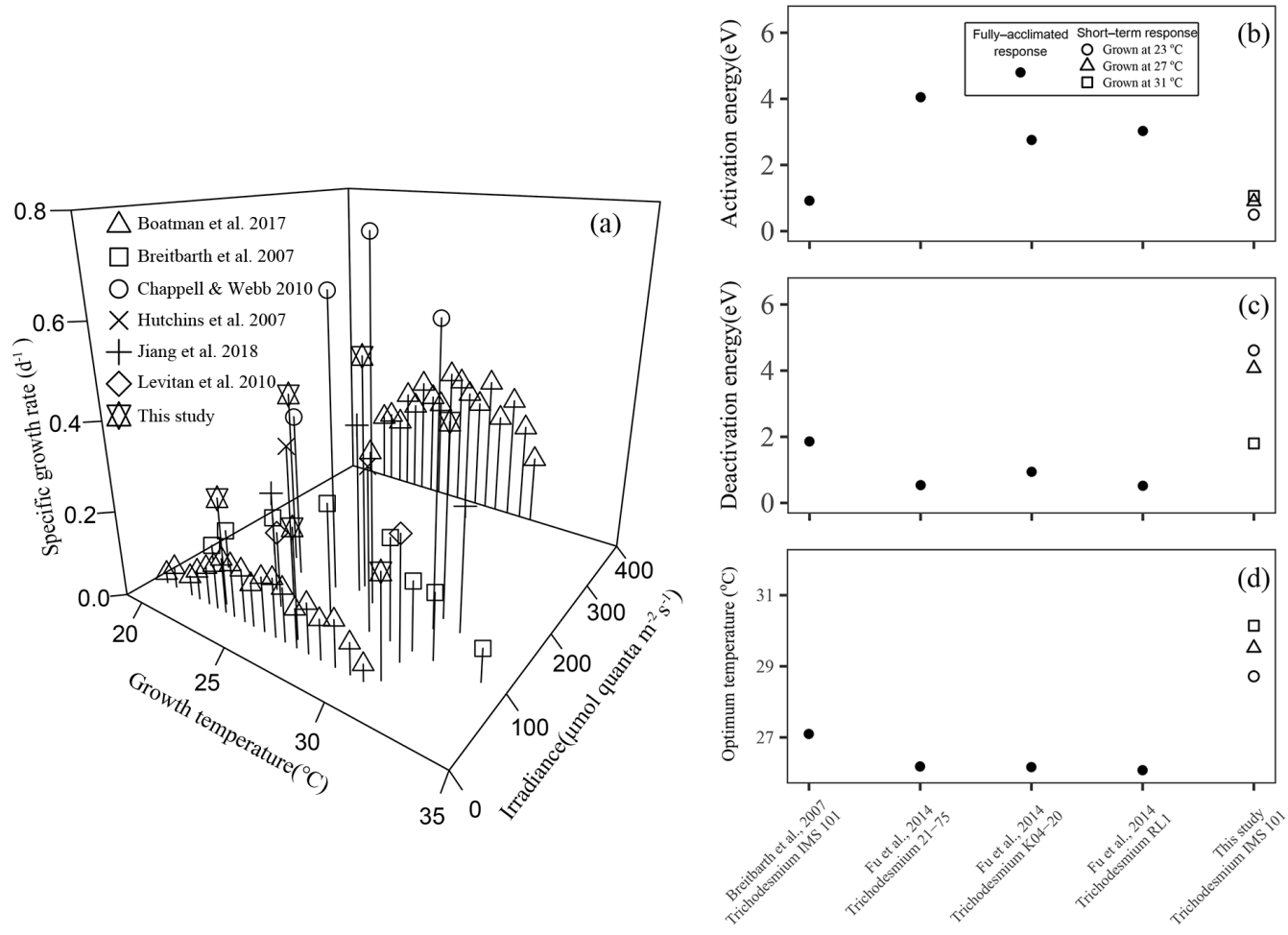

445 **Figure 5 (a) The combined effects of temperature and light intensity on the specific growth rate in *Trichodesmium* IMS 101; data from published literature involving at least two growth temperatures and this study. (b) Activation energy, (c) deactivation energy and (c) optimum temperature for N_2 fixation rate in *Trichodesmium*; data are calculated from published literature and this study; in the column of "This study", only light–saturated cultures are presented because of the similarity of growth light intensity to other two studies.**