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Effects of seawater acidification on the growth rates of the diatom *Thalassiosira (Conticribra) weissflogii* under different nutrient, light, and UV radiation regimes

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Abstract Effects of ocean acidification (OA) on marine organisms are suggested to be altered by other environmental drivers, such as low nutrient, increased light, and UVR exposures; however, little has been documented on this aspect. *Thalassiosira (Conticribra) weissflogii*, a marine diatom, was used to examine the OA effects under multiple stressors on its growth. The specific growth rate was inhibited by low nutrient (LN), though it increased with increased sunlight regardless of the nutrient supplies. Presence of UVR reduced the maximal growth rate (μ_{max}) in low CO_2 (LC) conditions (both LN and HN) and inhibited the apparent growth light use efficiency (α) in the cells acclimated to LN under both low (LC) and high (HC) CO_2 conditions. The HC-grown cells grew faster under HN and low light levels. Conclusively, presence of UVR with high solar radiation, LN and OA acted synergistically to reduce the diatom growth, though, in contrast UVR and OA enhanced the growth under HN.

Keywords Growth · Light intensity · Nutrient · Ocean acidification · *Thalassiosira (Conticribra) weissflogii* · UV radiation

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Introduction

The oceans are taking up anthropogenically released CO_2 at an average rate of 26 million tonnes per day, which remediates global warming but leads to ocean acidification (OA) (Gattuso et al. 2010). OA is known to have detrimental effects on calcification of most marine calcifying algae (Gao et al. 1993; Riebesell et al. 2000). However, the effects of OA on growth of diversified algal groups or species are controversial (Beardall et al. 2014). The OA effects on growth of diatoms can be stimulating, neutral, or inhibitory depending on species and/or environmental conditions as reported recently (Flynn et al. 2012; Gao and Campbell 2014).

Intracellular pH regulation is critical for cells to maintain their homoeostasis (Smith and Raven 1979). Increases in environmental H^+ concentration may raise energy requirements for intracellular pH regulation (Flynn et al. 2012), enhance photorespiration (Xu and Gao 2012), and increase mitochondrial-respiration (Wu et al. 2010; Yang and Gao 2012). On the other hand, increased concentrations of HCO_3^- and dissolved CO_2 may downregulate CO_2 concentration mechanisms (CCMs) with consequence of energy saving (Hopkinson et al. 2011). This may benefit some algal species in terms of enhanced growth rates (Gao et al. 2012b; Li et al. 2014). Therefore, the effects of OA on phytoplankton might depend on the energy balance between production and consumption during physiological processes. It has been suggested that, many other environmental variations such as light intensity, UVR, nutrient levels, and temperature could also modulate these effects indirectly (Beardall et al. 2009; Halac et al. 2010; Gao and Campbell 2014; Li et al. 2015; Passow and Laws 2015). For example, a recent study showed that the unicellular chlorophyte (*Dunaliella tertiolecta*) acclimated to elevated CO_2 under nutrient replete condition

could alleviate the stress induced by high photosynthetically active radiation (PAR) and UV (Garcia-Gomez et al. 2014).

Phytoplankters in natural waters are exposed to different levels of PAR and UVR doses due to distribution and weather conditions. With progressive global change, ocean warming is shoaling the thickness of up mixing layer, intensifying phytoplankton exposure to both UVR (280–400 nm) and PAR. Enhanced UV radiation, especially UVB, 280–315 nm, in the surface oceans has been suggested to induce inhibition of the photosynthesis processes of numerous phytoplankton taxa (Litchman and Neale 2005; Meador et al. 2009; Li et al. 2011), including the diatom *Thalassiosira weissflogii* (the currently accepted name is *Conticribra weissflogii*) (Stachura-Suchoples & Williams 2009; Halac et al. 2010). Because different intensities of solar UVR can result in differential effects on the primary production of phytoplankton (Gao et al. 2007), the interactive effects of light intensity and UVR combined with OA should be considered in the context of ocean global change biology. It has been previously shown that the growth of diatoms could be stimulated by increased pCO_2 when the light intensity and nutrient levels were optimal (Riebesell et al. 1993); however, OA reduced growth rates of several diatoms when grown under high levels of incident sunlight (Gao et al. 2012b). Enhanced susceptibility to UVR was found in the diatom *T. pseudonana* when acclimated to 1000 uatm CO_2 (Sobrino et al. 2008). Paradoxically, stimulating effects of OA on primary producers can differ spatial-temporally, especially in open oceans where cells live in stratified layers (Häder et al. 2015).

On the other hand, nutrient availability is considered the major limiting factor for primary production in most of the world's oceans (Tyrrell 1999; Beardall et al. 2001; Moore et al. 2001). Also, it is a determining factor that influences the effects of OA and UV on phytoplankton (Li et al. 2012a; Beardall et al. 2014; Flynn et al. 2015; Li et al. 2015). Negative effects of OA under nutrient-limited conditions have been suggested to be further amplified in the presence of high light and UVR exposure, presumably due to the nutrient dependency of photodamage-repairing processes (Litchman et al. 2002; Heraud et al. 2005; Mercado et al. 2014; Neale et al. 2014).

While the diatom *Skeletonema costatum* has been shown to be less affected by an acute pH drop after acclimation to elevated CO_2 (Zheng et al. 2015b), the impact of OA on diatoms is not fully understood, especially when multiple stressors are considered (Gao and Campbell 2014; Neale et al. 2014). This work tests the hypothesis that presence of UVR and high light under low nutrient conditions would further reduce the growth rate of diatom *T. weissflogii* grown in OA conditions.

Materials and methods

Biological material and pre-acclimation

Thalassiosira weissflogii (strain CCMA 102) was obtained from the Center for Collections of Marine Bacteria and Phytoplankton (CCMBP) located at State Key Laboratory of Marine Environmental Sciences (Xiamen University). The original isolate was from Dayawan Bay (China). Semi-continuous cultures were maintained by diluting the medium every 3 to 4 days; the cell density was controlled within a range of 50–5000 cells mL^{-1}. Cells were respectively grown at low (400 μatm, LC) and high (1000 μatm, HC) CO_2 levels, at 200 μmol photons m^{-2} s^{-1} with light: dark cycle of 12 h:12 h and 20 °C in 1 L polycarbonate bottles (Nalgene, USA). The CO_2 of LC and HC was acquired using the ambient air or mixture of pure CO_2 with ambient air in a CO_2 chambers (HP1000G-D, Ruihua Instrument & Equipment Co. Ltd., China). The CO_2 concentration was measured with a portable CO_2 meter (GM70, Vaisala CARBOCAP, Finland). Both LC and HC cultures were maintained for over 90 days, exceeding about 120 generations in standard Aquil medium (Morel et al. 1979). The nutrient levels of the medium were then modified to contain 50 μmol L^{-1} of NO_3^- and 5 μmol L^{-1} of PO_4^{3-} ('HN') or as low as 15 μmol L^{-1} of NO_3^- and 1.5 μmol L^{-1} of PO_4^{3-} ('LN', relative to the HN concentration) that with N/P ratio (10:1) less than the Redfield ratio (16:1), which could be found in coastal waters of South China Sea (Huang et al. 2015). All cells were acclimated under respective CO_2 and nutrient conditions for 20 generations before being used for subsequent experiments.

Outdoor culture set-up

The cells that were acclimated to indoor conditions as described above were transferred to natural irradiance conditions. The cells were cultured in 20-mL vials made of borosilicate glass (Perkin Elmer, USA); light transmission spectrum shown in Fig. 1. Cells were semi-continuously cultured, by partial dilution every 24 h with fresh media containing the corresponding CO_2 and nutrient levels. During the whole experiment period, the temperature was controlled with flowing-through water ranging from 25 to 27 °C during 1 day. The cell concentration ranged from 1000 to 5000 cells mL^{-1} during the experimental period, allowing the pH and carbonate system of LC and HC cultures to maintain stable conditions.

During outdoor culture, both light intensity and light quality (with or without UVR) were examined at two culture stages. For the first stage, the cells received 100 %, 37 %, 27 %, 17 %, or 10 % of incident solar irradiance (PAR + UVR) that was obtained by covering with different layers of neutral net. In order to avoid the potential damage or mortality

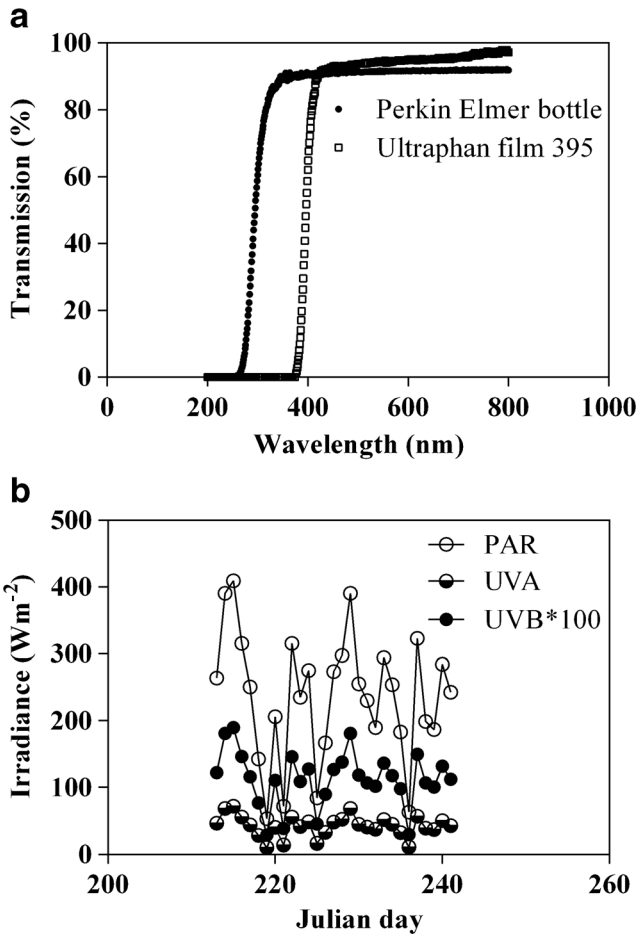

Fig. 1 Transmission spectrum of the culture bottles and UV-cutoff foil, Ultraphan film 395

due to the sudden shock by solar irradiance from indoor to outdoor transition, the cells were acclimated from 10 % of natural light (covered with five neutral net) step by step to higher light levels by removing one layer of neutral net each day until each of the set light intensity attained. The cells were acclimated under PAR + UVR for 18 days under a series of light intensities as described above, until the growth rates were calculated. To measure PAR, only Ultraphan film 395 (UV Opak, Digefra) was used. The light transmission is shown in Fig. 1. The Ultraphan film 395 was placed under the neutral net to remove the UVR and cells were acclimated for another 3 days, then the growth rate under series of PAR level as mentioned above was calculated. The actual light cells received inside the bottle were calculated according to the averaged transmission rate in different wavelength parts (PAR, UVB, and UVA band) of both the bottle and filter.

Growth rates

Cell concentration was evaluated microscopically using a hemocytometer. The specific growth rates (μ) of cells under different light, CO_2, and nutrient conditions were calculated according to the following equation:

$$\mu = (\ln N_2 - \ln N_1)/t$$

where N_1 and N_2 indicate the initial cell concentration and cell concentration after 24 h (t), respectively.

Irradiance

The solar radiation of photosynthetically active radiation (PAR) was measured with a QSL-2100 Scalar PAR irradiance sensor (Biospherical Instruments Inc., USA). PAR intensity was recorded at one-minute intervals, and the intensity of UVA and UVB was estimated according to the ratio of PAR to UVA and UVB that previously monitored with Eldonet broadband filter radiometer (Eldonet XP, Real Time Computer, Germany).

Statistical analyses

The apparent growth light use efficiency (α) of cell grown under different treatments were determined by assuming the growth rate was zero at zero light according to Gao et al. (2012b). Individual or interactive effects between CO_2 concentration, nutrient level, light intensity, and quality (with or without UVR) were analyzed using one- , two- , three- or four-way ANOVA to establish significant difference. Additionally, the Tukey HSD test was used for post-hoc comparisons. Paired/unpaired t test was used to analyze the significant difference between treatments. All the data were analyzed with Prism 5.0 and SPSS 16.0 software with the significance level of $p < 0.05$ ($n = 6$, each treatment has six replicate cultures).

Results

Solar irradiance and culture condition

The light transmission of the borosilicate glass bottle (Perkin Elmer, USA) was 16.3 to 81.9 % in UVB parts (280–320 nm) and 83.2 to 90.5 % in UVA parts (320–395 nm) (Fig. 1). During the duration of the experiment, the daily average light intensity of PAR ranged from 53.0 Wm^{-2} (242 μmol photons m^{-2} s^{-1}) to 409.4 Wm^{-2} (1870 μmol photons m^{-2} s^{-1}). Additionally, the daily averages of UVA and UVB were calculated, and they ranged from 10.38 to 72.08 Wm^{-2} and 0.28 to 1.90 Wm^{-2}, respectively (Fig.1b). During the days of growth rate calculation, the actual intensity of PAR, UVA, and UVB inside the bottle in PAR + UVR or PAR only treatments is shown in Table 1. The pH values of LC and HC samples were significantly different and the pH of LC ranged from 8.20 to 8.27 and HC pH ranged from 7.82 to 7.89

Table 1 Daily averaged PAR, UVA, and UVB intensity inside the bottle during the experiment days for growth rate calculation of with (+UVR) or without UVR (−UVR) treatments

		100 %	37 %	27 %	17 %	10 %
PAR + UVR	PAR (μmol photons m^{-2} s^{-1})	994.4 ± 220.1	367.9 ± 81.4	268.5 ± 59.4	169.0 ± 37.4	99.4 ± 22.0
	UVA (W m^{-2})	38.3 ± 6.8	14.2 ± 2.5	10.3 ± 1.8	6.5 ± 1.2	3.8 ± 0.7
	UVB*100 (W m^{-2})	64.4 ± 10.5	23.8 ± 3.9	17.4 ± 2.8	10.9 ± 1.8	6.4 ± 1.0
PAR	PAR (μmol photons m^{-2} s^{-1})	914.8 ± 292.0	338.5 ± 108.1	247.0 ± 78.8	155.5 ± 49.6	91.5 ± 29.2
	UVA (W m^{-2})	1.5 ± 0.3	0.6 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.2 ± 0.0
	UVB*100 (W m^{-2})	0	0	0	0	0

($p < 0.05$). The various light regimens did not alter the significance of these results (Table 2). Alternatively, no significant differences were observed among light treatments ($p > 0.05$).

Growth rate

Effects of OA on growth rate

Overall, there were no significant effects of OA on the growth rates of all treatments as indicated by four-way ANOVA analysis ($F_{1,80} = 0.202$, $p = 0.654$) (Table 3); however, when the environmental components are analyzed on an individual case, the response to OA seems to be effected. When cells were exposed to PAR + UVR, HC-HN cells were enhanced by 13.07 % (paired *t* test, $t = 2.817$, $p = 0.04$, df = 5) at PAR intensity of 17 %, 33.18 % (not significant, paired *t* test, $t = 1.865$, $p = 0.12$, df = 5) at a PAR intensity of 27 %, and 18.08 % (paired *t* test, $t = 3.086$, $p = 0.03$, df = 5) at PAR intensities of 37 % when compared to LC-HN treatments. No significant difference in growth rate was observed at 100 % PAR level (paired *t* test, $t = 1.520$, $p = 0.19$, df = 5) (Figs. 2a and 3a). Under HC-LN condition, significant inhibited growth rate compared with LC-LN cells, which decreased by 36.12 % (paired *t* test, $t = 7.528$, $p < 0.001$, df = 5), 17.66 % (paired *t* test, $t = 7.364$, $p < 0.001$, df = 5), and 29.67 % (paired *t* test, $t = 2.608$, $p = 0.048$, df = 5) at PAR of 10, 17, and 27 %, respectively (Figs. 2a and 3a). At light intensity of 100 %, growth rate of HC-cultured cells was significantly decreased by 19.77 % (paired *t* test, $t = 6.846$, $p = 0.001$, df = 5) compared with the LC-cultured cells (Figs. 2a and 3a).

When cells were cultured under PAR-only condition, different trends were observed compared to PAR + UVR-grown cultures (Fig. 2b). Under HN conditions, no significant differences were observed between LC and HC under low light condition, except for the light intensity of 17 % treatment that with 14.52 % (paired *t* test, $t = 3.234$, $p = 0.02$, df = 5) higher in LC group compared with HC one, where HC treatment showed significant inhibition of growth by 12.62 % (paired *t* test, $t = 6.154$, $p = 0.002$, df = 5) when exposed under 100 % of light intensity (Figs. 2b and 3b). Under LN treatments, compare to LC, HC significantly stimulated the growth rate by 23.16 % (paired *t* test, $t = 8.573$, $p < 0.001$, df = 5) at a light intensity of 37 %; however, higher light (100 %) significantly decreased the growth rate by 25.45 % (paired *t* test, $t = 15.080$, $p < 0.001$, df = 5) (Figs. 2b and 3b).

Effects of light intensity on growth rate

Both PAR + UVR (three-way ANOVA, $F_{4, 40} = 8.931$, $p < 0.001$) and PAR-only (three-way ANOVA, $F_{4, 40} = 42.110$, $p < 0.001$) conditions, the growth rates were stimulated with increased light intensity. Additionally, neither the CO_2 level nor the nutrient level altered the observed increase in growth rate (Fig. 2a). The growth rates (μ, day^{-1}) ranged from 1.042 ± 0.27 to 1.52 ± 0.21 in LC-HN, 1.12 ± 0.19 to 1.67 ± 0.14 in HC-HN, 0.63 ± 0.17 to 0.94 ± 0.24 in LC-LN, and 0.44 ± 0.11 to 1.03 ± 0.57 in HC-LN at series of light intensities (10 to 100 %) when grown under UV. When the UV irradiance was blocked, growth rates ranged from 0.91 ± 0.31 to 1.78 ± 0.17, 0.91 ± 0.17 to 1.55 ± 0.13,

Table 2 pH_{NBS} values of *T. weissflogii* grown under low (LC, 390 μatm) and high CO_2 (HC, 1000 μatm) conditions that enriched with high (NO_3^-, 50 μmol L^{-1}; PO_4^{3-}, 5 μmol L^{-1}) and low (NO_3^-, 15 μmol L^{-1}; PO_4^{3-}, 1.5 μmol L^{-1}) nutrient concentrations during the experiment. Data are represented as means ± SD of five measurements during the whole period of outdoor experimental days

	100 %	37 %	27 %	17 %	10 %
LC-HN	8.23 ± 0.04	8.25 ± 0.05	8.27 ± 0.06	8.24 ± 0.06	8.23 ± 0.06
LC-LN	8.24 ± 0.05	8.22 ± 0.03	8.23 ± 0.08	8.18 ± 0.06	8.20 ± 0.06
HC-HN	7.86 ± 0.04	7.86 ± 0.04	7.85 ± 0.05	7.87 ± 0.04	7.83 ± 0.05
HC-LN	7.86 ± 0.06	7.89 ± 0.06	7.82 ± 0.05	7.83 ± 0.08	7.86 ± 0.06

Table 3 Four-way ANOVA analysis of individual and interactive effects among CO_2 concentration (CO_2), nutrient level (*Nutrient*), light intensity (*LI*) and light quality (with or without UVR) on growth rate at $p < 0.05$ level

Treatments	*p* value	df	F	Significant
LI	<0.001	4	36.466	Yes
CO_2	0.654	1	0.202	No
Nutrient	<0.001	1	195.796	Yes
UVR	0.001	1	12.462	Yes
LI × CO_2	0.004	4	4.041	Yes
LI × Nutrient	0.283	4	1.270	No
LI × UVR	0.026	4	2.815	Yes
CO_2 × Nutrient	0.204	1	1.624	No
CO_2 × UVR	0.377	1	0.784	No
Nutrient × UVR	<0.001	1	14.585	Yes
LI × CO_2 × Nutrient	0.256	4	1.341	No
LI × CO_2 × UVR	0.694	4	0.557	No
LI × Nutrient × UVR	0.904	4	0.259	No
CO_2 × Nutrient × UVR	0.009	1	6.881	Yes
LI × CO_2 × Nutrient × UVR	0.455	4	0.917	No

0.68 ± 0.23 to 1.39 ± 0.14, and 0.63 ± 0.21 to 1.23 ± 0.21 in LC-HN, HC-HN, LC-LN, and HC-LN treatments, respectively.

Effects of UV on growth rate

As the experiment was carried out in two stages, with and without UVR treatments, and during the growth rate determination, the averaged light intensities of PAR (10 to 100 %) were basically no different in PAR + UVR or without UVR treatments; therefore, the comparison of UVR effects is meaningful. Comparison of growth rates with or without UVR indicates that UVR has a significant impact on overall growth rates (four-way ANOVA, $F_{1, 80} = 12.462$, $p < 0.001$). At light intensity of 10 % (108 μmol photons m^{-2} s^{-1} on average in both PAR + UVR and PAR), the growth rates were stimulated by UVR. UVR stimulated growth by 24.55 % (paired *t* test, $t = 4.547$, $p = 0.006$, df = 5) in LC-HN cells, 7.26 % (statistically not significant, paired *t* test, $t = 0.072$, $p = 0.946$, df = 5) in LC-LN, and 23.59 % (paired *t* test, $t = 8.506$, $p < 0.001$, df = 5) in HC-HN cells (Fig. 4a). Alternatively in HC-LN cells, UVR inhibited growth, by 28.30 % (paired *t* test, $t = 4.073$, $p = 0.010$, df = 5) (Fig. 4a). As the light intensity increased to near saturation point (37 %, 400 μmol photons m^{-2} s^{-1} on average), UVR-induced growth inhibition decreased as indicated by the lack of statistically significant differential growth rates. LC-HN cells had a 9.64 % (statistically not significant, paired *t* test, $t = 2.270$, $p = 0.073$, df = 5) reduction; LC-LN cells had a 11.19 % (paired *t* test, $t = 3.386$, $p = 0.020$, df = 5) reduction, and HC-LN cells had a 19.47 % (statistically not significant, paired *t* test, $t = 1.235$, $p = 0.272$, df = 5) reduction. However, existing of UVR stimulated the growth rate of HC-HN by 11.70 % (paired *t* test, $t = 6.079$, $p = 0.002$, df = 5) (Fig. 4b). At full intensity of solar irradiance (100 %, 1080 μmol photons m^{-2} s^{-1} on average), the inhibition induced by UVR was 14.78 % (paired *t* test, $t = 7.500$, $p < 0.001$, df = 5), 33.23 % (paired *t* test, $t = 8.825$, $p < 0.001$, df = 5), 7.34 % (statistically not significant, paired *t* test, $t = 1.335$, $p = 0.240$, df = 5), and 28.37 % (paired *t* test, $t = 4.911$, $p = 0.004$, df = 5) in LC-HN, LC-LN, HC-HN, and HC-LN treatments, respectively. Irrespective of low, saturated, or full solar irradiance, UVR-induced inhibition was significantly different among treatments (one-way ANOVA, all $p < 0.05$) (Fig. 4).

The maximum growth rate (μ_{max}) and calculated apparent growth light use efficiency (α) are shown in Table 4. In general, UVR and LN significantly affected the μ_{max} and α individually and interactively (three-way ANOVA, all $p < 0.05$). No significant difference of μ_{max} was detected in either light condition when cells were cultured in HC conditions (both HN and LN) (unpaired *t* test, all $p > 0.05$); however, the

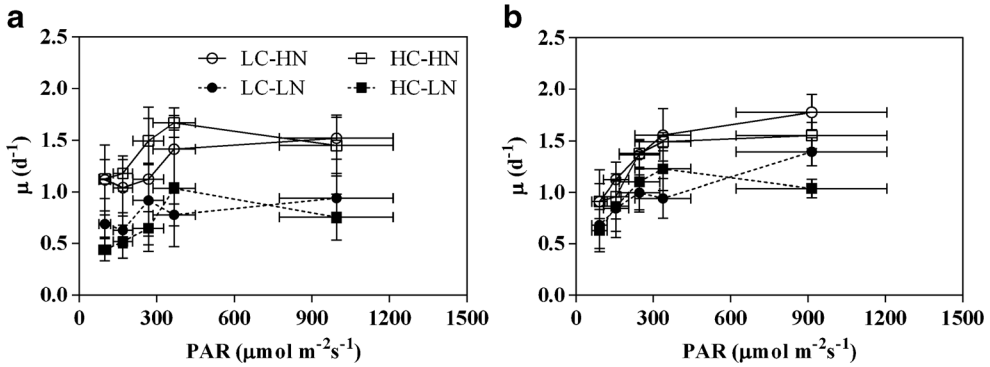

Fig. 2 Growth rates of *T. weissflogii* grown under low (*LC*, 390 μatm) and high CO_2 (*HC*, 1000 μatm) conditions that enriched with high (NO_3^-, 50 μmol L^{-1}; PO_4^{3-},5 μmol L^{-1}) and low (NO_3^-,15 μmol L^{-1}; PO_4^{3-}, .5 μmol L^{-1}) nutrient concentrations, **a** irradiance with PAR + UVR (>295 nm), after then, **b** the Ultraphan film 395 was covered to remove off UVR (>395 nm) under solar radiation. Each treatment has six replicate cultures and the data are represented as mean ± SD, $n = 6$

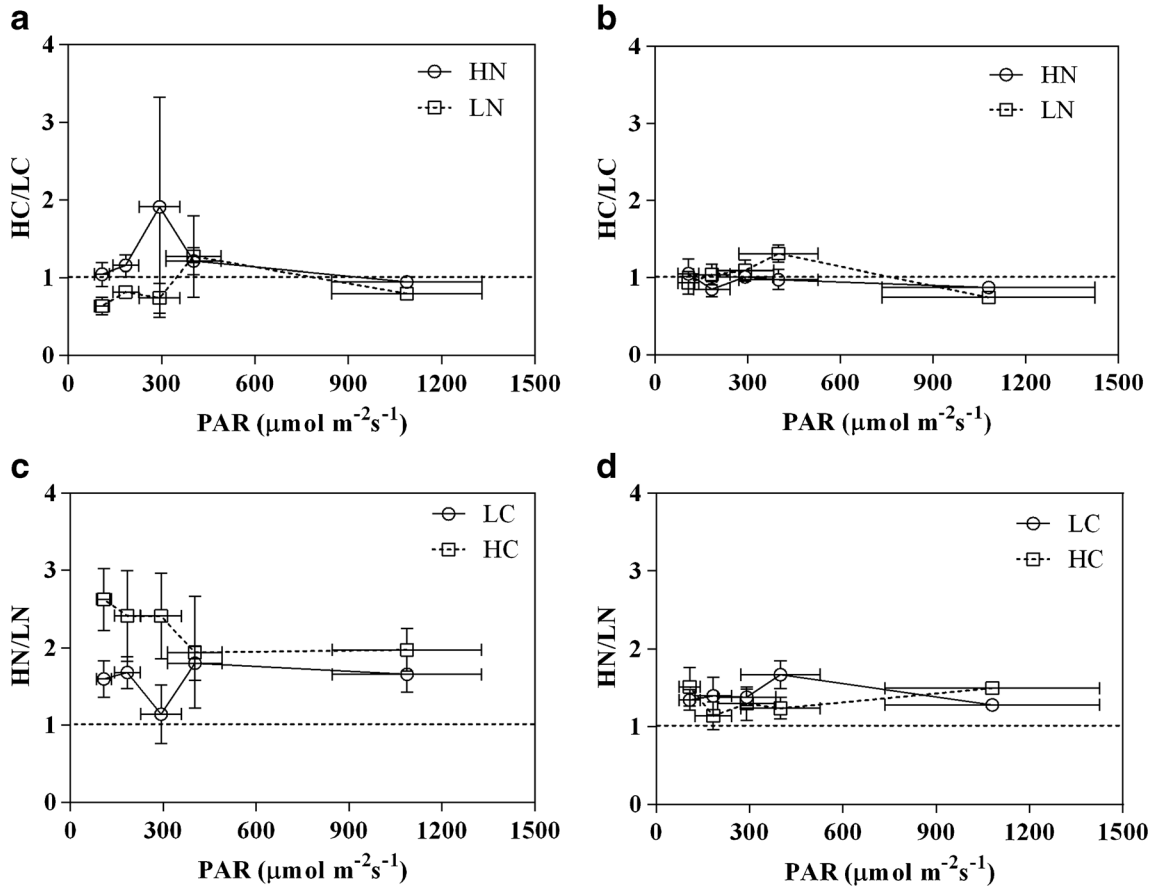

Fig. 3 The growth rates ratio of **a**, **b** HC to LC and **c**, **d** HN to LN of *T. weissflogii* grown under low (*LC*, 390 μatm) and high CO_2 (*HC*, 1000 μatm) conditions that enriched with high (NO_3^-, 50 μmol L^{-1}; PO_4^{3-},5 μmol L^{-1}) and low (NO_3^-,15 μmol L^{-1}; PO_4^{3-}, 1.5 μmol L^{-1}) nutrient concentrations (**a**, **c**) with (>295 nm) or (**b**, **d**) without UVR (>395 nm). Each treatment has six replicate cultures and the data were represented as mean ± SD, $n = 6$

μ_{max} was higher under LC conditions (both HN and LN) when the UVR was removed (LN, by 47.87 %, unpaired *t* test, $t = 4.041$, $p = 0.002$, df = 10; HN, by 17.11 %, unpaired *t* test, $t = 2.394$, $p = 0.037$, df = 10) (Table 4). No significant difference of α in cells exposed to or not exposed to UVR was observed under HN conditions; however, under LN conditions, HC and LC cells showed an increase of 42.86 % (unpaired *t* test, $t = 2.285$, $p = 0.045$, df = 10) and 41.38 % (unpaired *t* test, $t = 3.336$, $p = 0.008$, df = 10) when UVR was removed (Table 4).

Effects of nutrients on growth rate

Low nutrient supply significantly decreased the growth rates of all treatments (four-way ANOVA, $F_{1,\ 80} = 195.80$, $p < 0.001$) (Table 3). Under HC conditions, the higher HN/LN ratio suggested cells acclimated to PAR + UVR at low light intensities (10 %: unpaired *t* test, $t = 5.423$, $p = 0.0003$, df = 10; 17 %: unpaired *t* test, $t = 2.890$, $p = 0.0161$, df = 10; 27 %: unpaired *t* test, $t = 4.639$, $p = 0.0009$, df = 10) (Fig. 3c). A lowered ratio of HN/LN was observed at 100 % light level in LC-grown cells when acclimated without UVR (unpaired *t* test, $t = 6.751$, $p < 0.001$, df = 10) (Fig. 3d). The HN/LN ratio was mediated by UVR (three-way ANOVA, $F_{1,100} = 89.48$, $p < 0.001$) and CO_2 concentration (three-way ANOVA, $F_{1,100} = 28.29$, $p < 0.001$) individually and interactively (three-way ANOVA, $F_{1,100} = 28.29$, $p < 0.001$) (Fig. 3c, d).

Coupling effect

According to the statistically supported observations of this experiment, light intensity, UVR, and nutrient level all have a significant effect on the growth rate of *T. weissflogii* when examined individually (all $p < 0.05$) (Table 3). There was also a coupled effect when between light intensity and CO_2, light intensity and UVR, nutrient level and UVR, the two factors, and among CO_2, nutrient level, and UVR, the three factors were evidenced (all $p < 0.05$) (Table 3).

Discussion

In the current scenario (business as usual) of global environmental change, primary production of phytoplankton may not

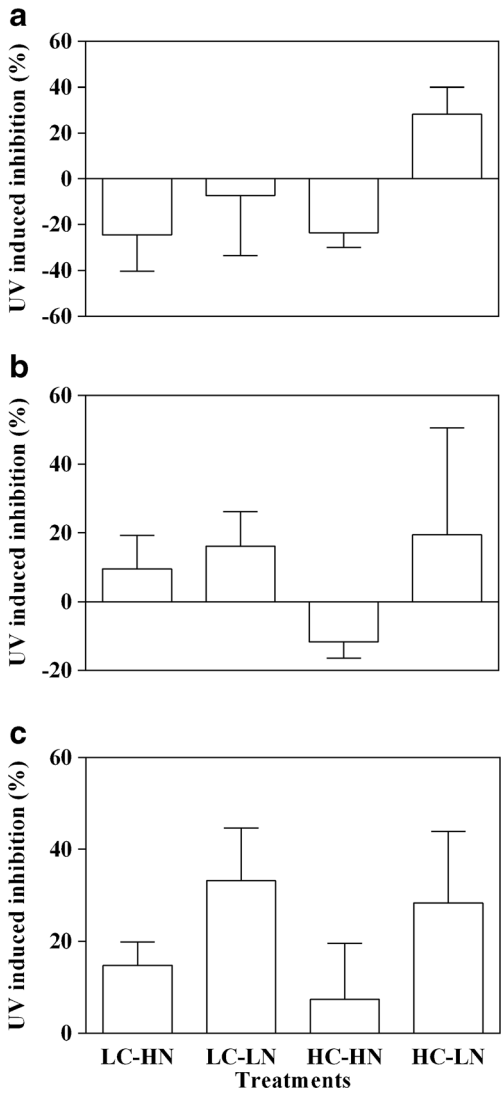

Fig. 4 Inhibition of growth rates due to UVR on *T. weissflogii* under sunlight of **a** 10 %, **b** 37 % and **c** 100 %, $n = 6$

Table 4 The maximum growth rate (μ_{max}, day^{-1}) and calculated apparent growth light use efficiency (α) of *T. weissflogii* grown under low (LC, 390 μatm) and high CO_2 (HC, 1000 μatm) conditions that enriched with low (NO_3^-, 15 μmol L^{-1}; PO_4^{3-}, 1.5 μmol L^{-1}) and high (NO_3^-, 50 μmol L^{-1}; PO_4^{3-}, 5 μmol L^{-1}) nutrient concentrations during the experiment

		μ_{max}	α
LC-HN	+UVR	1.52 ± 0.21^a	0.045 ± 0.015^a
	−UVR	1.78 ± 0.17^b	0.049 ± 0.007^a
LC-LN	+UVR	0.94 ± 0.24^a	0.029 ± 0.007^a
	−UVR	1.39 ± 0.14^b	0.041 ± 0.006^b
HC-HN	+UVR	1.67 ± 0.14^a	0.054 ± 0.006^a
	−UVR	1.55 ± 0.13^a	0.053 ± 0.005^a
HC-LN	+UVR	1.03 ± 0.57^a	0.028 ± 0.011^a
	−UVR	1.23 ± 0.21^a	0.040 ± 0.007^b

Different superscript letters indicate significant difference between the treatments with (+UVR) or without UVR (−UVR)

respond to ocean acidification (OA) only but have to be modulated by numerous environmental forcing (Häder and Gao 2015). There are dominating drivers in determining the final effects (Brennan and Collins 2015). In this study, main forces to influence primary production (Gao et al. 2012b; Beardall et al. 2014), OA, low nutrient, and high light and UV exposures, acted synergistically to reduce growth of the diatom *T. weissflogii*.

The effects of OA are recognized as potentially having profound influences on marine primary producers. However, this topic is highly controversial because of effects reported in different species under different culture conditions (Turley et al. 2010). The species-specific physiological traits to OA perhaps could be an explanation (eg. different affinity to CO_2); in addition, OA does not affect algae performance (biological, physiological, metabolic, and trophic cascade processes) alone, and different experimental designs can lead to controversial results under different conditions (light intensity, nutrient concentrations, UVR, temperature etc.) (Mercado et al. 2014; Neale et al. 2014; Reul et al. 2014; Sobrino et al. 2014; Sobrino et al. 2008;). Response of algae to OA has been shown to vary under different levels or combinations of the factors (see the reviews by Beardall et al. (2014), Gao and Campbell (2014) and Riebesell and Tortell (2011), and references therein).

Under high CO_2 conditions, most phytoplankton show decreased affinity of photosynthesis for dissolved inorganic carbon (DIC) and reflect a downregulated carbon concentrating mechanism (CCM) (Giordano et al. 2005; Roberts et al. 2007; Raven et al. 2008). CCM down regulation has been suggested to save about 20 % energy in several diatoms including *T. weissflogii* (Hopkinson et al. 2011). However, the net effects of saved energy may depend on other variables (Gao et al. 2012a; Li et al. 2012a; Shi et al. 2012; Hennon et al. 2014; Zheng et al. 2015a). In the present study, when given full spectrum of solar radiation, increased growth rates were only found in cells grown under high-nutrient and low PAR conditions (Figs. 2a, 3a), but growth rates were inhibited under low nutrient condition (Figs. 2a and 3a). The predicted shortage of nutrient supply in future ocean ecosystems due to intensified stratification could induce decreased growth of pelagic phytoplankton (Caraco et al. 1990; Beardall et al. 2009; Beardall et al. 2014). While high light and UVR have great inhibitory effects on nutrient and carbon assimilation mechanisms (Helbling and Zagarese 2003), the lowered nutrient load may further increase UV-induced inhibition of photosynthesis and growth (Beardall et al. 2014). Meanwhile, low nutrient could lead to a decrease of both photoprotection pigments (UV screen capacity) and repair processes (energy synthesis, D1 protein, etc.) (Litchman et al. 2002; Beardall et al. 2009). However, *T. weissflogii* is often found in coastal waters, where

nutrients are rarely limiting, therefore, the impacts of the multiple stressors for this species would differ in different waters.

Compared with the HN-grown cells, growth rates of LN-treated cells significantly decreased in both LC and HC cultures, regardless of exposure with or without UVR (Fig. 3c, d). While the LN level (relative to the HN) in this study may not traditionally considered as nutrient limitation as reported in previous studies (Laws and Bannister 1980; Caperon and Meyer 1972; Garside and Glover 1991; Laws et al. 2013), the reduced availability of nutrient in LN did significantly lowered the growth rate. There is a possibility that nutrient limitation levels, even for the same species, change according to light levels or due to differences in diurnal fluctuation of sunlight. Furthermore, the nitrogen quota in this diatom could be higher under the higher levels of temperature and light in the present work than reported by Laws and Bannister (1980) (maximum of 27 pg cell^{-1}). Nevertheless, maintaining the cell concentration less than 5000 per mL would only change nitrate concentration by less than 20 % in HN but by 65 % in LN if estimated using the N quota of 27 pg cell^{-1}. Therefore, change in nitrate levels under the treatments can be considered very small.

High levels of PAR and UVR may damage the energy dissipation system and cause higher inhibition (Gao et al. 2007; Sobrino et al. 2008). Nutrient limitation, specifically nitrogen, has been shown to synergistically act with UVB to inhibit photosynthesis in *D. tertiolecta* and enhance the susceptibility of the cells to UV-induced overall damage, though stimulated repair rates were also found in nitrogen-limited condition (Shelly et al. 2002). In the present study, decreased in α under LN were further lowered in the presence of UVR. Therefore, it was speculated that a reduced nutrient supply limited the potential ability of cells to utilize the saved energy under HC conditions, especially when UVR and high PAR levels were present, which leads to further growth inhibitions. However, cells exposed to 100 % PAR of natural irradiance showed either no change (HN) or decreased (LN) growth rates under HC conditions, suggesting high PAR and UVR could act synergistically with lower nutrient supply to down regulate the growth of cells in OA conditions. This is not surprising because it is generally accepted that low nutrient supply has the potential to exacerbate the susceptibility of phytoplankton to OA and UVR (Beardall et al. 2009, 2014).

No significant differences in growth rates were observed between LC and HC under HN conditions at the low PAR level (without UVR) (Fig. 2b); HC treatment appeared to stimulate the growth of phytoplankton under low PAR condition (Gao et al. 2012b). However, the enhanced mitochondria respiration, photorespiration, and the acid base balance modulation could enhance the energy consumption concurrently (Wu et al. 2010; Li et al. 2012a; Xu and Gao 2012), which may counteract the positive effects of OA and result in no stimulation of growth rate, as shown here in *T. weissflogii*. HC decreased the growth rate in both LN and HN conditions under high-light (100 %) treatments, which supports previously reported data (Gao et al. 2012b) that HC could synergistically interact with increased light exposure to reduce the growth rate of primary producers.

UVR is considered to have many inhibitory effects on physiological performances of micro and macro-algae, such as damaging the molecules, suppressing the carbon and nutrients assimilation, stimulating the reactive oxygen species (ROS), and reducing the primary production (Häder et al. 2007; Segovia et al. 2015). On the other hand, low levels of UVA or UVB have been shown to play a role in repairing UV-induced damage and, in some cases, stimulating photosynthesis and photoprotective mechanisms (Helbling et al. 2003; Gao et al. 2007; Hanelt and Roleda 2009; Xu and Gao 2010). However, the positive effects of UV, sometimes, are intensity-dependent (Shelly et al. 2003). For example, a former study has demonstrated that the "double-edged sword theory" of UVR that it could stimulate the photosynthetic performance in low but depress it in high-PAR condition (Gao et al. 2007). In the present study, UVR exposure promoted growth rate of HN-cultured cells under low PAR (10 % light intensity level) condition supporting the positive effects of UVR in low PAR environments, consistent with the previous reported results (Gao et al., 2007). The unaffected μ_{max} of HC-grown cells showed higher resistance to UV radiation, which accords with the findings that ocean acidification could remit the UVB-related photochemical inhibition and help in repairing UV-induced damage (Li et al. 2012b; Garcia-Gomez et al. 2014). However, in LN-cultured cells, growth rates were decreased in all light conditions in both LC- and HC-treated cells with UVR exposure and with the lowest μ_{max} in LC-LN treatment. These results may further extend the previously suggested hypothesis that the "double-edged sword" of UVR is a nutrient-dependent process.

In conclusion, the results presented here indicate that exposure to UVR could mediate the growth response of *T. weissflogii* to OA and light intensity. Additionally, low nutrient supply could act synergistically with OA to further down regulate the growth rate of cells in high light and/or UVR conditions. The statistically supported observed differential responses of growth rates to OA under different nutrient levels, broad range of light intensities and UVR emphasize the importance and necessity of a multi-factor study when global change effects are being evaluated.

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