Future CO_2-induced seawater acidification mediates the physiological performance of a green alga *Ulva linza* in different photoperiods

Furong Yue[1], Guang Gao[1], Jing Ma[1], Hailong Wu[1], Xinshu Li[1] and Juntian Xu[1,2,3]

[1] Jiangsu Key Laboratory of Marine Bioresources and Environment, Huaihai Institute of Technology, Lianyungang, China

[2] Co-Innovation Center of Jiangsu Marine Bio-industry Technology, HuaiHai Institute of Technology, Lianyungang, China

[3] Jiangsu Key Laboratory of Marine Biotechnology, Huaihai Institute of Technology, Lianyungang, China

ABSTRACT

Photoperiods have an important impact on macroalgae living in the intertidal zone. Ocean acidification also influences the physiology of macroalgae. However, little is known about the interaction between ocean acidification and photoperiod on macroalgae. In this study, a green alga *Ulva linza* was cultured under three different photoperiods (L: D = 8:16, 12:12, 16:8) and two different CO_2 levels (LC, 400 ppm; HC, 1,000 ppm) to investigate their responses. The results showed that relative growth rate of *U. linza* increased with extended light periods under LC but decreased at HC when exposed to the longest light period of 16 h compared to 12 h. Higher CO_2 levels enhanced the relative growth rate at a L: D of 8:16, had no effect at 12:12 but reduced RGR at 16:8. At LC, the L: D of 16:8 significantly stimulated maximum quantum yield (Yield). Higher CO_2 levels enhanced Yield at L: D of 12:12 and 8:16, had negative effect at 16:8. Non-photochemical quenching (NPQ) increased with increasing light period. High CO_2 levels did not affect respiration rate during shorter light periods but enhanced it at a light period of 16 h. Longer light periods had negative effects on Chl *a* and Chl *b* content, and high CO_2 level also inhibited the synthesis of these pigments. Our data demonstrate the interactive effects of CO_2 and photoperiod on the physiological characteristics of the green tide macroalga *Ulva linza* and indicate that future ocean acidification may hinder the stimulatory effect of long light periods on growth of *Ulva* species.

Subjects Marine Biology, Climate Change Biology
Keywords Growth, Ocean acidification, Photoperiod, Photosynthesis, *Ulva linza*

INTRODUCTION

Due to the activities of humans, the concentration of atmospheric CO_2 has increased to 400 ppm from 278 ppm during the pre-industrial revolution (*Gattuso et al., 2015*). It is estimated that the oceans, as a CO_2 sink, have taken up approximately 48% of the fossil-fuel and cement-manufacturing emissions (*Sabine et al., 2004*). The concentration of atmospheric CO_2 has been predicted to reach almost 1,000 ppm by the end of 21st

century (*Gattuso et al., 2015*). This would result in the decline of surface ocean pH by 0.3–0.4 units, an increase of hydrogen ion concentration and bicarbonate concentration in seawater, which is termed ocean acidification (OA) (*Orr et al., 2005*; *Koch et al., 2013*; *Albright, 2016*). OA will be maintained for hundreds of years, and thus could cause a huge change in marine ecosystems (*Kroeker et al., 2013*; *Wittmann & Pörtner, 2013*).

Marine phytoplankton accounts for 50% of global primary production (*Beardall, Stojkovic & Larsen, 2009*) and macroalgae accounts for 10% of marine primary productivity (*Smith, 1981*; *Charpy-Roubaud & Sournia, 1990*; *Heinrich et al., 2012*). Macroalgae have evolved distinct strategies to capture inorganic carbon (Ci) for photosynthesis: (1) by the diffusive uptake of CO_2 (*Giordano, Beardall & Raven, 2005*; *Raven et al., 2005*), (2) the active transport of HCO_3^-/CO_2 (termed carbon concentrating mechanisms (CCMs) (*Maberly, Raven & Johnston, 1992*; *Raven, 2003*; *Giordano, Beardall & Raven, 2005*). The elevated CO_2 might enhance the growth and photosynthesis of algae that depend on CO_2 diffusion or have weak CCMs (*Kübler, Johnston & Raven, 1999*; *Gao et al., 2016a*). Therefore, the physiological influences of OA on macroalgae might be species-specific. For example, OA has a positive influences on the growth of macroalgae, such as the red algae *Pyropia yezoensis, Pyropia haitanensis* and *Gracilaria lemaneiformis* (*Gao et al., 1991*; *Xu & Gao, 2015*; *Chen et al., 2018*), green algae *Ulva prolifera, Ulva lactuca, Caulerpa taxifolia* (*Xu & Gao, 2012*; *Chen, Zou & Jiang, 2015*; *Roth-Schulze et al., 2018*) and brown algae *Hizikia fusiforme, Ecklonia radiata, Sargassum muticum* (*Zou, 2005*; *Britton et al., 2016*; *Xu et al., 2017*). However, research has shown that high CO_2 levels did not have a detectable effect on the relative growth rate of several species, for example, *Sargassum henslowianum, Ulva rigida, Ulva australis*, the giant kelp *Macrocystis pyrifera* and *Ulva australis* (*Chen & Zou, 2014*; *Rautenberger et al., 2015*; *Fernández, Roleda & Hurd, 2015*; *Reidenbach et al., 2017*). Furthermore, increasing seawater CO_2 concentration reduced the growth of *Gracilaria tenuistipitata*, **Porphyra leucosticte**, *Fucus vesiculosus, Halimeda opuntia* and *Ulva linza* (*García-Sánchez, Fernández & Niell, 1994*; *Mercado et al., 1999*; *Gutow et al., 2014*; *Johnson, Price & Smith, 2014*; *Gao et al., 2018*). In addition, the various effects of ocean acidification may be also modulated by other environmental factors, such as temperature, nutrients and photoperiods (*Gao et al., 2017a*; *Gao et al., 2019*; *Li et al., 2018*).

Photoperiod is a key factor regulating seasonal responses of algae (*Dring, 1988*). Furthermore, daylength can influence algal affinity for dissolved inorganic carbon and cellular carbon demand, thus imposing an effect on CCMs regulation (*Rost, Riebesell & Sültemeyer, 2006*). Thus, the photoperiod may be critical for algae cultured under elevated CO_2 levels. Meanwhile, the interactive effects of OA and increased photoperiods could enhance the growth of *U. prolifera*, thus increase the opportunities for occurrence of green tides (*Li et al., 2018*). Elevated CO_2 levels enhanced the photosynthetic rate, but longer illumination periods reduced the photosynthetic efficiency in *E. huxleyi* (*Nielsen, 1997*; *Rost, Riebesell & Sültemeyer, 2006*). Therefore, at HC, the responses of algae to different photoperiods appear to be genera- and species-specific.

Green macroalgal bloom has occurred each year in the Yellow Sea (YS) since 2007, termed green tides (*Xu, Zhang & Cheng, 2016*). Green tides generate aesthetic problems and toxic gas when thalli decompose (*Gao et al., 2016b*). *Ulva* is the dominant genus

contributing majority of green tides and *U. linza* is one of species causing green tides in the Yellow Sea (*Fletcher, 1996*; *Kang et al., 2014*; *Gao et al., 2017a*; *Gao et al., 2017b*). However, little is known about the interactive effects of ocean acidification and photoperiod on *U. linza*. Previous study showed that the effect of ocean acidification on diatoms was related to light intensity (*Gao et al., 2012*). In this study, based on previous studies, we hypothesized the effect of ocean acidification on *U. linza* may dependent on photoperiod. To test our hypothesis, the physiological responses of the green macroalga *Ulva linza* under two different CO_2 levels and three different photoperiods were examined.

MATERIALS & METHODS

Thalli collection and culture conditions

U. linza was collected from the coastal water of Gaogong peninsula (119.3°E, 34.5°N), Lianyungang, Jiangsu Province, China. Gaogong peninsula is a public place and no approval is required for collecting naturally growing *Ulva* species in China because *Ulva* species cause green tides in coastal waters of the Yellow Sea. *U. linza* was identified by morphological characters (*Ma et al., 2009*). The samples were transferred to the laboratory in a portable cooler (4–6 °C) box within one hour. Healthy thalli (first observed by color and then checked with maximum quantum yield of PSII) were selected and cleaned with filtered natural seawater to remove sediments, visible epiphytes and attached animals. Thalli were pre-cultured in a 500 ml flask in an illuminated incubator (GXZ-500B, Ningbo, China) at 20 °C, with the illumination intensity set at 150 μmol photons m^{-2}s^{-1} (12L:12D). Sterilized filtered seawater (salinity 30, supplied with 8 μM NaH_2PO_4 and 60 μM $NaNO_3$) was used as culture medium and the medium was bubbled with air before being renewed every two days.

For these experiments, approximately three 1 cm long thalli were placed into a 550 ml flask filled with 500 ml sterile seawater aerated ambient outdoor air (400 ppm, LC) or with CO_2-enriched air (1,000 ppm, HC) and randomly cultured in three separate incubators with different light levels (8L:16D, 12L:12D, 16L:8D) and continuous aeration at 20 °C with 150 μmol photons m^{-2}s^{-1}. Different CO_2 levels (400 ppm, LC; 1,000 ppm, HC) were achieved by bubbling ambient air or CO_2-enriched air via a CO_2 plant incubator (HP 1000 G-D, Ruihua Instruments, Wuhan, China).

To maintain the pH_{NBS} at about 8.12 (LC) and 7.78 (HC) under different photoperiods, the increased algal biomass were removed constantly and the medium was changed every two days and daily variations in pH were maintained at less than 0.05. The experiment lasted 9 days, the physiological factors were taken on the last 3 days.

Estimate of carbonate system parameters

The seawater pH was monitored with a pH meter (pH 700, Eutech Instruments, Singapore) and total alkalinity (TA) was calculated by titrations (*Gao et al., 2019*). Other parameters of the carbonate system were obtained with CO2SYS software (*Pierrot, Lewis & Wallace, 2006*), the equilibrium constants K_1 and K_2 for carbonic acid dissociation (*Roy et al., 1993*).

Measurement of growth

The length of *U. linza* was recorded every 2 days. The relative growth rate (RGR) of thalli were calculated across the 6-day period before other physiological parameters were measured during the following 3 days. RGR was calculated as follows: $RGR = \ln(W_t/W_0)/t$, where W_t is the length after t days culture, W_0 is the initial length.

Chlorophyll fluorescence measurements

Chlorophyll *a* fluorescence in *U. linza* was measured with a portable PAM (Pulse-amplitude-modulation; AquaPen-P AP-P 100, Chech). Algae were dark adapted for 15 min before the experiment. The parameters were calculated according to the following equations:

$rETR = PAR \times Y(II) \times 0.84 \times 0.5$ (*Schreiber, 2004*; *Zhang, Zhang & Yang, 2017*),

where rETR is the relative electron transport rate; PAR is the actinic light; Y(II) is the effective quantum yield of PSII.

$NPQ = Fm - Fm'/1$ (*Bilger & Schreiber, 1986*),

where NPQ is the non-photochemical quenching; Fm is the maximum fluorescence value of *U. linza* when they were adapted in the dark for 15 min; Fm' is the maximum fluorescence value of *U. linza* under actinic light conditions.

Respiration rate measurements

The respiration rate of *U. linza* was measured using a Clark-type oxygen electrode (YSI Model 5300, Yellow Springs Instrument Co., USA). The thallus was cut into 1 cm long segments with scissors and the thalli were placed in culture conditions for at least 1 h to decrease the effects of cutting damage. Approximately 0.01 g fresh weight of thalli were placed in the reaction chamber with 8 ml medium. Temperature was controlled at 20 °C with a circulating water bath. The decrease of the oxygen content in the seawater in darkness with seven minutes was defined as the respiration rate.

Measurement of photosynthetic pigments

Chlorophyll *a* and *b* were extracted from thalli (about 10 mg FW) with 5 ml methanol at 4 °C for 24 h in the dark. The absorption values were obtained at 652 nm, 663 nm and 665 nm using an ultraviolet spectrophotometer (Ultrospect 3300 pro; Amersham Bioscience, Sweden). The contents of the Chl *a* and Chl *b* were estimated using the method of *Porra, Thompson & Kriedemann (1989)*.

Data analysis

All the data are shown as mean ± SD. Origin 9.0 and SPSS 18.0 were used to analyze data. Two-way ANOVA was used to assess the interactive effects of CO_2 levels and photoperiods on relative growth rate, chlorophyll fluorescence parameters, respiration rate and pigment content of *U. linza*. One-way ANOVA was used to analyze differences under the same conditions. Tukey HSD was conducted for *post hoc* investigation. Confidence intervals were set at 95%.

Table 1 Parameters of the seawater carbonate system in different cultures.

	pH_{NBS}	pCO_2 (μatm)	DIC (μmol kg^{-1})	HCO_3^- (μmol kg^{-1})	CO_3^{2-} (μmol kg^{-1})	CO_2 (μmol kg^{-1})	TA (μmol kg^{-1})
8:16 LC	8.14 ± 0.02[a]	580.32 ± 35.74[a]	2597.04 ± 57[a]	2385.19 ± 56.15[a]	192.59 ± 7.23[a]	19.56 ± 1.19[a]	2850.78 ± 56.26[a]
12:12 LC	8.12 ± 0.01[a]	613.13 ± 11.21[a]	2594.32 ± 61.96[a]	2391.03 ± 55.85[a]	182.94 ± 6.07[a]	20.35 ± 0.37[a]	2834.31 ± 67.99[a]
16:8 LC	8.13 ± 0.01[a]	608.54 ± 14.83[a]	2638.26 ± 40.24[a]	2427.94 ± 35.09[a]	190.12 ± 6.73[a]	20.19 ± 0.49[a]	2886.86 ± 46.67[a]
8:16 HC	7.74 ± 0.03[b]	1614.77 ± 100.05[b]	2780.28 ± 12.05[b]	2641.71 ± 12.23[b]	84.99 ± 5.08[b]	53.58 ± 3.32[b]	2854.16 ± 13.32[a]
12:12 HC	7.79 ± 0.02[cd]	1445.67 ± 58.75[c]	2799.47 ± 14.61[b]	2655.70 ± 13.74[b]	95.80 ± 3.81[b]	47.97 ± 1.95[c]	2893.15 ± 17.18[a]
16:8 HC	7.75 ± 0.01[bd]	1652.45 ± 43.38[b]	2938.18 ± 47.63[c]	2790.86 ± 45.52[c]	92.48 ± 1.28[b]	54.83 ± 1.44[b]	3019.14 ± 46.95[b]

Data are the mean ± SD ($n = 3$).
DIC, dissolved inorganic carbon; TA, total alkalinity.

RESULTS

Both the elevated CO_2 levels and photoperiod altered carbonate parameters in seawater, and they both had an interactive effect (Tables 1 and 2). The elevated CO_2 decreased pH and CO_3^{2-}, increased pCO_2, DIC, HCO_3^- and CO_2 in the seawater. Increased photoperiod did not affect carbonate parameters at LC but the longest photoperiod increased DIC, HCO_3^- and TA compared to shortest photoperiod.

The two-way ANOVA showed that elevated CO_2 and photoperiod had an interactive effect, and both elevated CO_2 levels and the photoperiods had a significant effect on the RGR of *U. linza* (Fig. 1 and Table 3). At LC, the RGR of adult *U. linza* increased with the extended light periods, and the highest RGR occurred at a L: D of 16:8. The effect of CO_2 also varied with photoperiod. Higher CO_2 levels enhanced RGR at L: D of 8:16, but had no effect at 12:12 and reduced it at 16:8.

The Yield and NPQ were measured under different CO_2 levels and photoperiod conditions (Fig. 2). Two-way ANOVA showed that elevated CO_2 and photoperiod had an interactive effect on Yield (Table 4). Higher CO_2 levels increased Yield when thalli were cultured under photoperiods of 8:16 and 12:12 but reduced it under 16:8. Photoperiod had the main effect on NPQ (Table 4). At LC, thalli cultured at L: D of 16:8 had higher NPQ compared to L: D of 8:16 while the difference between 8:16 and 12:12 was insignificant. At HC, NPQ increased with the increase in photoperiod although the increase was not statistically significant. The elevated CO_2 had neutral effect on NPQ of *U. linza*.

Maximum rETR (rETRmax), efficiency of electron transport (α), and saturating irradiance (I_k) were calculated from the rapid light curves (Fig. 3, Table 5). Photoperiod and elevated CO_2 levels had an interactive effect, and elevated CO_2 levels had a main effect on light-saturated electron transport rate (rETRmax) (Table 6). Higher CO_2 levels increased rETRmax during the 8:16 and 12:12 photoperiods, but did not affect it at 16:8. A similar pattern was also found for α. In contrast to rETRmax and α, CO_2 did not affect I_k while photoperiod had the main effect on it. At LC, I_k increased when L: D rose from 8:16 to 12:12 but did not change with the further increase in photoperiod. At HC, I_k did not change when L: D rose from 8:16 to 12:12 but was enhanced when L: D increased to 16:8 (Table 5).

Table 2 Two-way ANOVA analysis of variance for the effects of CO_2 and photoperiod regimes on pH, pCO_2, dissolved inorganic carbon (DIC), HCO_3^-, CO_3^{2-}, CO_2 and total alkalinity (TA) in seawater.

Source	df	pH		pCO_2		DIC		HCO_3^-		CO_3^{2-}		CO_2		TA	
		F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.
Photoperiod	2	1.333	0.3	5.640	0.019	9.697	0.003	10.112	0.003	.353	0.710	5.632	0.019	8.638	0.005
CO_2	1	2268.750	<0.001	1498.294	<0.001	125.390	<0.001	236.530	<0.001	1453.669	<0.001	1497.372	<0.001	8.977	0.011
Photoperiod*CO_2	2	7.750	0.007	7.562	0.007	3.054	0.085	3.184	0.078	5.342	0.022	7.557	0.008	2.976	0.089
Error	12														

Notes.

CO_2*photoperiod means the interactive effects of CO_2 and photoperiod, df mean degree of freedom and F means the value of F statistic, and Sig. means *p*-value.
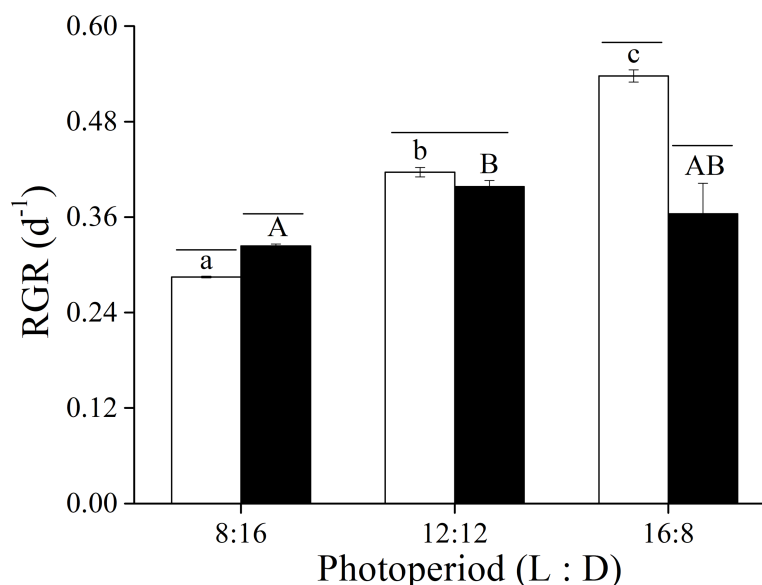
Figure 1 **Relative growth rate.** Relative growth rate (RGR) of *U. linza* thalli grown at different CO_2 levels (400 ppm, LC; 1000 ppm, HC) and different photoperiods (L: D = 8:16, 12:12, 16:8).

Full-size ⬜ DOI: 10.7717/peerj.7048/fig-1

Table 3 **Two-way ANOVA analysis of variance for the effects of CO_2 and photoperiod regimes on RGR of *Ulva linza* cultured at different CO_2 levels and photoperiod regimes.**

Source	df	F	Sig.
Photoperiod	2	124.249	<0.001
CO_2	1	42.292	<0.001
Photoperiod* CO_2	2	65.959	<0.001
Error	12		

Notes.
CO_2*photoperiod means the interactive effects of CO_2 and photoperiod, df mean degree of freedom and F means the value of F statistic, and Sig. means *p*-value.

In addition to photosynthetic parameters, the effects of p CO_2 and photoperiod on the respiration rate of adult *U. linza* were also investigated (Fig. 4). Photoperiod and elevated CO_2 levels had an interactive effect, and both photoperiod and elevated CO_2 levels had the primary effect on the respiration rate of *U. linza* (Table 7). Higher CO_2 levels did not affect the respiration rate at photoperiods of 8:16 or 12:12 but increased it by 56.39% at a photoperiod of 16:8.

Changes in photosynthetic pigments of *U. linza* grown under various conditions are shown in Fig. 5. Two-way ANOVA showed that CO_2 and photoperiod had an interactive effect, and both CO_2 levels and the photoperiods had the main effect on the Chl *a* content of *U. linza* (Table 8). Prolonged light periods reduced the synthesis of Chl *a* in thalli although the difference between the photoperiods of 8:16 and 12:12 at HC was not statistically significant. Higher CO_2 levels reduced Chl *a* at the photoperiods of 8:16 and 12:12 but did not affect it at a L: D of 12:12. The same trend was found for Chl *b*. The Chl *a/b* ratios were all greater than 1.0, suggesting a higher synthesis of Chl *a* than Chl *b* under all culture
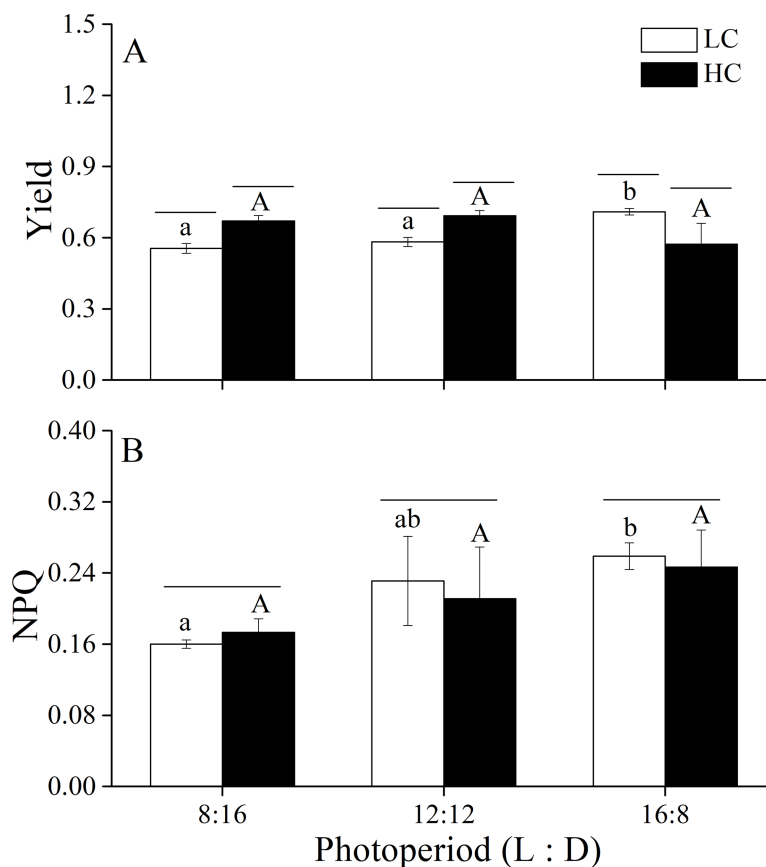
Figure 2 **Yield and NPQ.** Yield (A) and NPQ (B) of *U. linza* thalli grown at different CO_2 levels (400 ppm, LC; 1000 ppm, HC) and different photoperiods (L: D = 8:16, 12:12, 16:8).

Full-size ⊡ DOI: 10.7717/peerj.7048/fig-2

Table 4 **Two-way ANOVA analysis of variance for the effects of CO_2 and photoperiod regimes on Yield and NPQ of *Ulva linza* cultured at different CO_2 levels and photoperiod regimes.**

Source	Yield			NPQ		
	df	F	Sig.	df	F	Sig.
Photoperiod	2	0.919	0.425	2	8.453	0.005
CO_2	1	2.549	0.136	1	0.132	0.722
Photoperiod* CO_2	2	19.786	<0.001	2	0.339	0.719
Error	12			12		

Notes.
CO_2*photoperiod means the interactive effects of CO_2 and photoperiod, df mean degree of freedom and F means the value of F statistic, and Sig. means *p*-value.

conditions. Photoperiod and elevated CO_2 levels had an interactive effect on the Chl *a/b* ratio (Table 8); the higher CO_2 levels increased the ratio at a photoperiod of 12:12 but not at the other photoperiods.

Yue et al. (2019), *PeerJ*, DOI 10.7717/peerj.7048

8/19
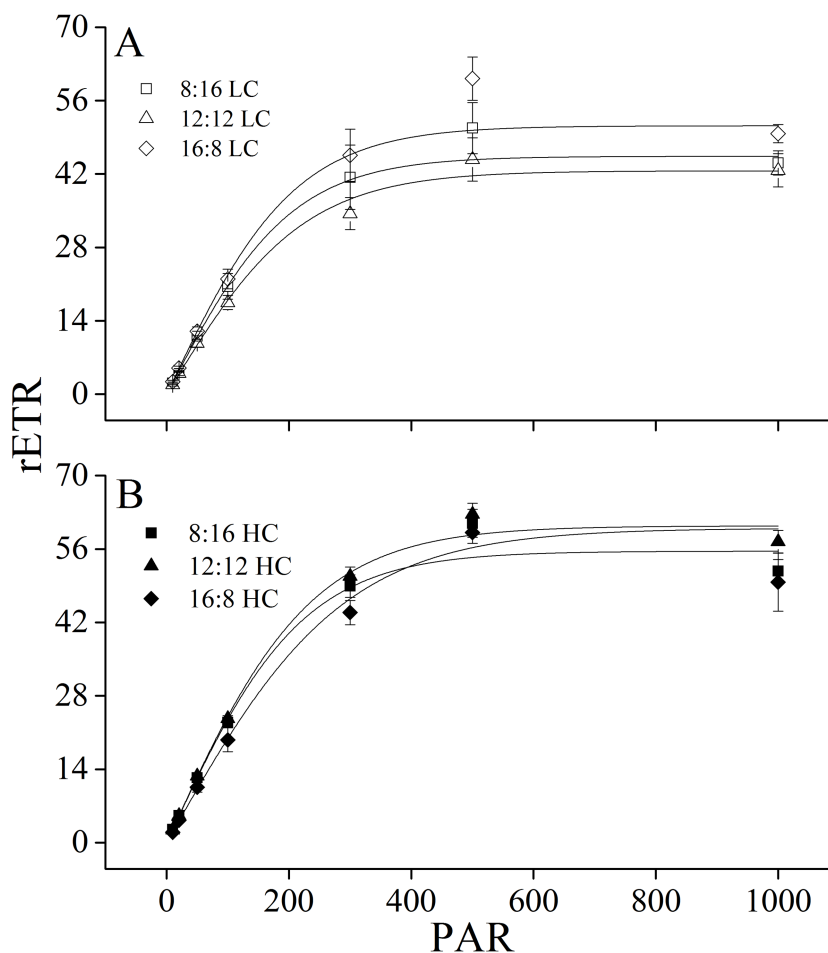
Figure 3 Rapid light curves. Rapid light curves (RLC) of *U. linza* thalli grown at different CO_2 levels (400 ppm, LC; 1,000 ppm, HC) and different photoperiods (L: D = 8:16, 12:12, 16:8).

Full-size 🖼 DOI: 10.7717/peerj.7048/fig-3

Table 5 Parameters of rapid light curves (RLC) of *U. linza* thalli cultured at different CO_2 levels and photoperiod regimes.

	rETRmax	α	I_k
8:16 LC	47.53 ± 3.63[a]	0.22 ± 0.04[a]	215.67 ± 19.89[a]
12:12 LC	43.87 ± 3.60[c]	0.18 ± 0.02[c]	248.87 ± 22.91[bd]
16:8 LC	54.83 ± 2.65[b]	0.24 ± 0.03[ab]	231.58 ± 21.37[adef]
8:16 HC	56.51 ± 2.34[b]	0.25 ± 0.01[b]	222.15 ± 13.41[ac]
12:12 HC	60.45 ± 1.38[d]	0.26 ± 0.01[b]	236.58 ± 12.63[bce]
16:8 HC	54.55 ± 3.77[b]	0.22 ± 0.01[a]	251.72 ± 11.69[bf]

Data are the mean ± SD ($n \geq 3$). Different letters represent significant difference ($p < 0.05$) among different treatments.
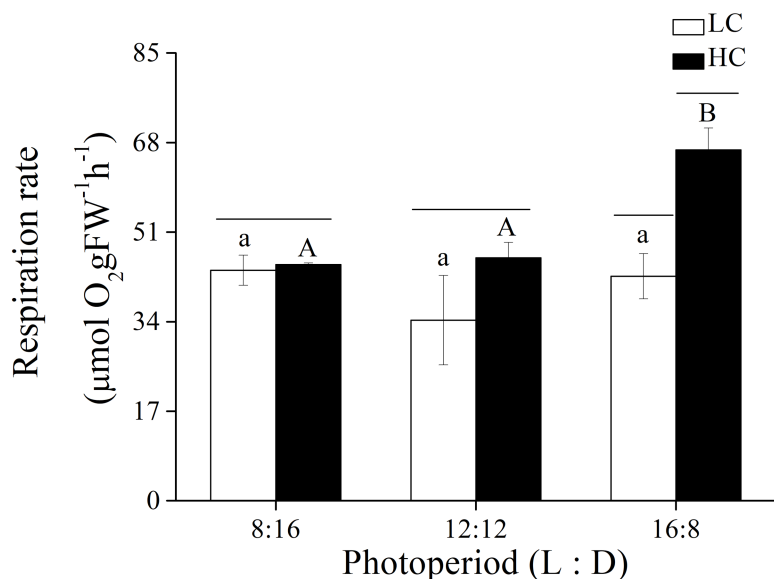
Figure 4 Respiration rate. Respiration rate of *U. linza* thalli grown at different CO_2 levels (400 ppm, LC; 1,000 ppm, HC) and different photoperiods (L: D = 8:16, 12:12, 16:8).

Full-size ☐ DOI: 10.7717/peerj.7048/fig-4

Table 6 Two-way ANOVA analysis of variance for the effects of CO_2 and photoperiod regimes on maximum rETR (rETRmax), efficiency of electron transport (α), and saturating irradiance (I_k) of *Ulva linza* cultured at different CO_2 levels and photoperiod regimes.

Source	rETRmax			α			I_k		
	df	F	Sig.	df	F	Sig.	df	F	Sig.
Photoperiod	2	2.981	0.066	2	2.909	0.070	2	7.030	0.003
CO_2	1	69.993	<0.001	1	15.225	<0.001	1	0.664	0.421
Photoperiod* CO_2	2	23.445	<0.001	2	14.896	<0.001	2	2.576	0.093
Error	30			30			30		

Notes.
CO_2*photoperiod means the interactive effects of CO_2 and photoperiod, df mean degree of freedom and F means the value of F statistic, and Sig. means *p*-value.

Table 7 Two-way ANOVA analysis of variance for the effects of CO_2 and photoperiod regimes on respiration rate of *Ulva linza* cultured at different CO_2 levels and photoperiod regimes.

Source	df	F	Sig.
Photoperiod	2	16.006	<0.001
CO_2	1	33.058	<0.001
Photoperiod* CO_2	2	9.566	0.003
Error	12		

Notes.
CO_2*photoperiod means the interactive effects of CO_2 and photoperiod, df mean degree of freedom and F means the value of F statistic, and Sig. means *p*-value.
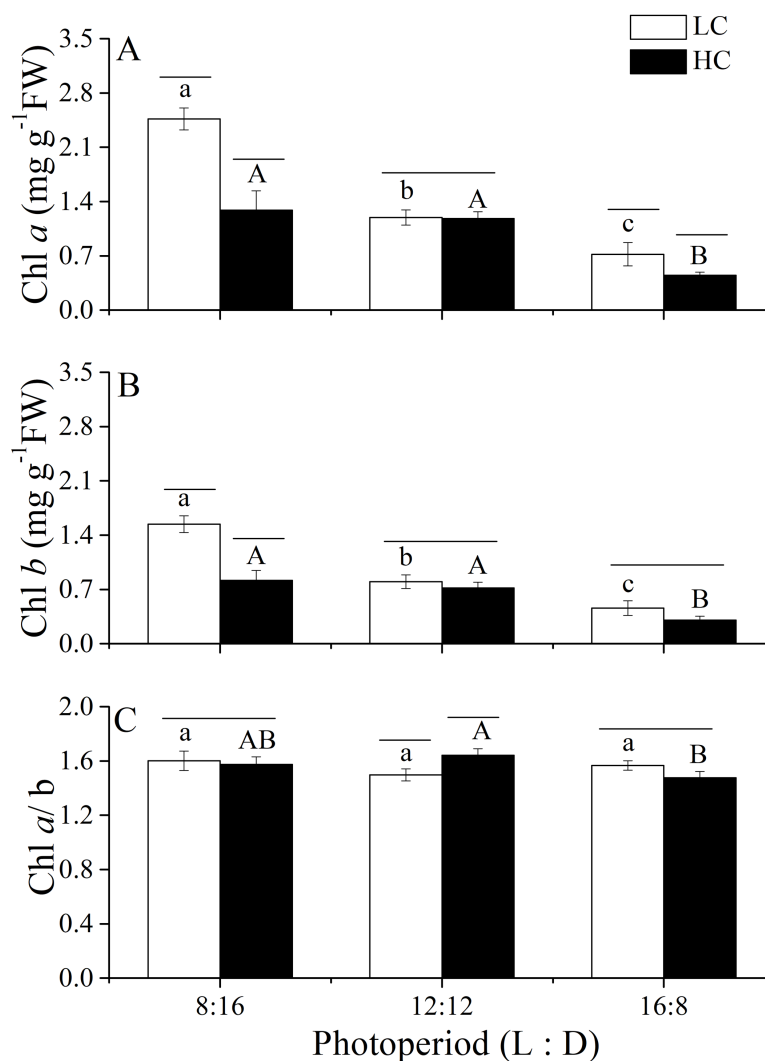
Figure 5 **Pigment contents and the Chl a./b ratio.** Pigment contents (Chl a, (A); Chl b, (B)) and the Chl a/b ratio (C) of *U. linza* thalli grown at different CO_2 levels (400 ppm, LC; 1,000 ppm, HC) and different photoperiods (L: D = 8:16, 12:12, 16:8).

Full-size ◙ DOI: 10.7717/peerj.7048/fig-5

DISCUSSION

In the present study, at LC, extended photoperiods had a positive effect on the relative growth rate of adult *U. linza,* similar to previous studies on *Laminaria sacharina, Porphyra umbilicalis* and *Ulva prolifera* (*Fortes & Lüning, 1980; Green & Neefus, 2016; Li et al., 2018*). Carbon isotope fractionation experiments suggested that extended photoperiods could enhance growth by influencing inorganic carbon capture and fixation rate in algae (*Rost et al., 2003*). This hypothesis is supported by the present study where extended photoperiods increased the maximum quantum yield in PS II. On the other hand, the highest growth rates of *Compsopogon coeruleus* were obtained in shorter light periods (L: D = 8:16) (*Zucchi & Necchi, 2001*), the highest growth rates of *Porphyra umbilicalis* was found under neutral

Table 8 Two-way ANOVA analysis of variance for the effects of CO_2 and photoperiod regimes on Chl *a*, Chl *b*, and Chl *a/b* of *Ulva linza* cultured at different CO_2 levels and photoperiod regimes.

Source	Chl *a*			Chl *b*			Chl *a/b*		
	df	F	Sig.	df	F	Sig.	df	F	Sig.
Photoperiod	2	121.283	<0.001	2	111.035	<0.001	2	2.625	0.113
CO_2	1	51.793	<0.001	1	53.849	<0.001	1	0.171	0.686
Photoperiod* CO_2	2	26.932	<0.001	2	21.719	<0.001	2	8.389	0.005
Error	12			12			12		

Notes.

CO_2*photoperiod means the interactive effects of CO_2 and photoperiod, df mean degree of freedom and F means the value of F statistic, and Sig. means *p*-value.

(L: D = 12:12) and longer (L: D = 16:8) light periods using 110 µmol photons $m^{-2}s^{-1}$ (*Green & Neefus, 2016*), and the relative growth rate of *Chlorella vulgaris* cultured at L: D = 20:4 was lower than the RGR of alga cultured at L: D = 16:8 (*Kendirlioglu, Agirman & Cetin, 2015*). Therefore, the effects of photoperiod on algae appear to be species-specific.

Although *Ulva* has efficient mechanisms for CO_2 concentration, the growth of *Ulva* can be enhanced by elevated CO_2 (*Young & Gobler, 2016*; *Gao et al., 2016a*; *Gao et al., 2017a*; *Gao et al., 2017b*). However, in this study, we found that the effects of elevated CO_2 levels on the relative growth rate of *U. linza* depended on photoperiod. CO_2 enhanced the growth of adult *U. linza* under light/dark conditions of 8:16, had no effect on growth of *U. linza* under a L: D of 12:12, and reduced the relative growth rate under a L: D of 16:8. High CO_2 levels can down-regulate algal CO_2 concentration mechanisms (CCMs), meaning that energy would be saved, and thus enhancing the relative growth rate of algae (*Gao et al., 2012*; *Gao et al., 2016a*; *Raven, Beardall & Sánchez-Baracaldo, 2017*). This is supported by decreased pigment synthesis in thalli at higher CO_2 levels. However, higher CO_2 levels did not affect the growth rate of *U. linza* at medium photoperiods. The neutral effects of CO_2 on the growth of *U. rigida* (*Rautenberger et al., 2015*) and *U. linza* (*Gao et al., 2018*) were also reported. We speculate that this is a compromise between the positive effects of elevated CO_2 and negative effects of decreased pH. The negative effect of decreased pH on growth was documented for the brown alga *Sargassum muticum* (*Xu et al., 2017*). Algae might need to consume additional energy to act against acid–based perturbation caused by decreased pH, leading to reduced growth (*Xu et al., 2017*). This is supported by an enhanced respiration rate at the higher CO_2 levels in this study. The phenomenon of an increased respiration rate of algae under elevated CO_2 concentrations was found in *Hizikia fusiformis* (*Zou, 2005*), the microalgae *Phaeodactylum tricornutum* (*Wu, Gao & Riebesell, 2010*) and *Emiliania huxleyi* (*Jin et al., 2015*).

Furthermore, the higher CO_2 level reduced the growth rate of *U. linza* under the longest photoperiod in the present study. This may be due to the combination of down-regulated CCMs and excess light energy. The operation of CCMs is an energy-consuming process and the down-regulation of CCMs can result in additional energy (*Raven, Beardall & Sánchez-Baracaldo, 2017*). Higher light can usually reduce algal photosynthetic activity (*Singh & Singh, 2015*; *Gao et al., 2016a*). The energy saved due to down-regulation of CCMs at HC combined with high light intensity could synergistically damage the algal

photosystem and photosynthetic rate (*Gao et al., 2012*; *Gao et al., 2016a*). Although light intensity did not change among different CO_2 treatments, the lengthened photoperiod may have similar effect to increased light intensity. This argument is supported by increased NPQ at longer photoperiods as NPQ is photo-protective process to dissipate excess light energy. Higher CO_2 levels also reduced maximum quantum yield and stimulated the respiration rate of *U. linza* during the longest photoperiod in this study, leading to the decrease in growth. Enhanced respiration rate is a signal that organisms are fighting against stress and damage (*Xu et al., 2017*).

In addition to growth, the interactive effect of CO_2 and photoperiod was also found in photosynthetic parameters. For instance, elevated CO_2 increased Yield, rETRmax and α at shorter photoperiods (L: D of 8:16 and 12:12) but did not affect rETRmax and reduced Yield at longest photoperiod (L: D of 16:8). These findings indicate the close connection between growth and photosynthesis in terms of responding to the combination of CO_2 and photoperiod. It is worth noting that the interaction of CO_2 and photoperiod on growth of *U. linza* in this study is different from the findings in *U. prolifera* reported by *Li et al. (2018)*. Elevated CO_2 increased growth of *U. prolifera* at all three photoperiods (L: D of 12:12, 10:14 and 16:8). The different results may be due to differential physiological property between *U. linza* and *U. prolifera*. It has been documented that *U. prolifera* has a higher tolerance to high light intensity compared to *U. linza* (*Cui et al., 2015*). The strong capacity in dealing with high light intensity could contribute to the effect of elevated CO_2 and prolonged photoperiod on growth *U. linza* was still positive. Integrating our findings with *Li et al. (2018)* study, the interaction of CO_2 and photoperiod on *Ulva* species would be species dependent.

CONCLUSIONS

This work is the first attempt to clarify the interaction between light/dark and elevated CO_2 levels on the physiological responses of *Ulva linza*. We found that the effect of OA on *U. linza* depended on photoperiod. Outbreaks of green tides during spring and summer in China occur when the photoperiod is reaching its peak. Our findings indicate future OA may hinder the occurrence of green tides dominated by *U. linza* in combination with extended photoperiods. More environmental factors, such as temperature and nutrient levels, need to be investigated to obtain a more comprehensive understanding on development of green tides in future oceans.

ADDITIONAL INFORMATION AND DECLARATIONS

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Furong Yue conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Guang Gao conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, approved the final draft.
- Jing Ma performed the experiments, analyzed the data, authored or reviewed drafts of the paper, approved the final draft.
- Hailong Wu approved the final draft.
- Xinshu Li analyzed the data, contributed reagents/materials/analysis tools, approved the final draft.
- Juntian Xu conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

U. linza was collected from the coastal water of Gaogong peninsula (119.3°E, 34.5°N), Lianyungang, Jiangsu Province, China. Gaogong peninsula is a public place and no approval is required for collecting naturally growing Ulva species in China because Ulva species cause green tides in coastal waters of the Yellow Sea.

Data Availability

The following information was supplied regarding data availability:

The raw data are available in Data S1.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.7048#supplemental-information.

REFERENCES

Albright R. 2016. Reversal of ocean acidification enhances net coral reef calcification. *Nature* **531**:362–365 DOI 10.1038/nature17155.

Beardall J, Stojkovic S, Larsen S. 2009. Living in a high CO_2 world: impacts of global climate change on marine phytoplankton. *Plant Ecology & Diversity* **2**:191–205 DOI 10.1080/17550870903271363.

Bilger W, Schreiber U. 1986. Energy-dependent quenching of dark-level chlorophyll fluorescence in intact leaves. *Photosynthesis Research* **10**:303–308 DOI 10.1007/bf00118295.

Britton D, Cornwall CE, Revill AT, Hurd CL, Johnson CR. 2016. Ocean acidification reverses the positive effects of seawater pH fluctuations on growth and photo-synthesis of the habitat-forming kelp, *Ecklonia radiata*. *Scientific Reports* **6**:26036 DOI 10.1038/srep26036.

Charpy-Roubaud C, Sournia A. 1990. The comparative estimation of phytoplanktonic, microphytobenthic and macrophytobenthic primary production in the oceans. *Marine Microbial Food Webs* **4**:31–57.

Chen B, Zou D. 2014. Growth and photosynthetic activity of Sargassum henslowianum (Fucales, Phaeophyta) seedlings in responses to different light intensities, tem-peratures and CO_2 levels under laboratory conditions. *Marine Biology Research* **10**:1019–1026 DOI 10.1080/17451000.2013.872798.

Chen B, Zou D, Du H, Ji Z. 2018. Carbon and nitrogen accumulation in the economic seaweed *Gracilaria lemaneiformis* affected by ocean acidification and increasing temperature. *Aquaculture* **482**:176–182 DOI 10.1016/j.aquaculture.2017.09.042.

Chen B, Zou D, Jiang H. 2015. Elevated CO_2 exacerbates competition for growth and photosynthesis between *Gracilaria lemaneiformis* and *Ulva lactuca*. *Aquaculture* **443**:49–55 DOI 10.1016/j.aquaculture.2015.03.009.

Cui J, Zhang J, Huo Y, Zhou L, Wu Q, Chen L, Yu K, He P. 2015. Adaptability of free-floating green tide algae in the Yellow Sea to variable temperature and light intensity. *Marine Pollution Bulletin* **101**:660–666 DOI 10.1016/j.marpolbul.2015.10.033.

Dring MJ. 1988. Photocontrol of development in algae. *Annual Review of Plant Physiology and Plant Molecular Biology* **39**:157–174 DOI 10.1146/annurev.pp.39.060188.001105.

Fernández PA, Roleda MY, Hurd CL. 2015. Effects of ocean acidification on the photosynthetic performance, carbonic anhydrase activity and growth of the giant kelp *Macrocystis pyrifera*. *Photosynthesis Research* **124**:293–304 DOI 10.1007/s11120-015-0138-5.

Fletcher RL. 1996. The occurrence of ''green tides''—a review. In: *Marine benthic vegetation*. Berlin: Springer, 7–43 DOI 10.1007/978-3-642-61398-2_2.

Fortes MD, Lüning K. 1980. Growth rates of North Sea macroalgae in relation to temperature, irradiance and photoperiod. *Helgoländer Meeresuntersuchungen* **34**:15–29 DOI 10.1007/BF01983538.

Gao K, Aruga Y, Asada K, Ishihara T, Akano T, Kiyohara M. 1991. Enhanced growth of the red alga Porphyra yezoensis Ueda in high CO_2 concentrations. *Journal of Applied Phycology* **3**:355–362 DOI 10.1007/bf02392889.

Gao G, Beardall J, Bao M, Wang C, Ren W, Xu J. 2018. Ocean acidification and nutrient limitation synergistically reduce growth and photosynthetic performances of a green tide alga Ulva linza. *Biogeosciences* **15**:3409–3420 DOI 10.5194/bg-15-3409-2018.

Gao G, Clare AS, Rose C, Caldwell GS. 2017a. Eutrophication and warming-driven green tides (*Ulva rigida*) are predicted to increase under future climate change scenarios. *Marine Pollution Bulletin* **114**:439–447 DOI 10.1016/j.marpolbul.

Gao G, Gao Q, Bao M, Xu J, Li X. 2019. Nitrogen availability modulates the effects of ocean acidification on biomass yield and food quality of a marine crop *Pyropia yezoensis*. *Food Chemistry* **271**:623–629 DOI 10.1016/j.foodchem.2018.07.090.

Gao G, Liu Y, Li X, Feng Z, Xu J. 2016a. An ocean acidification acclimatized green tide alga is robust to changes of seawater carbon chemistry but vulnerable to light stress. *PLOS ONE* **11**:e0169040 DOI 10.1371/journal.pone.0169040.

Gao G, Liu Y, Li X, Feng Z, Xu Z, Wu H, Xu J. 2017b. Expected CO_2-induced ocean acidification modulates copper toxicity in the green tide alga Ulva prolifera. *Environmental and Experimental Botany* **135**:63–72 DOI 10.1016/j.envexpbot.2016.12.007.

Gao K, Xu J, Gao G, Li Y, Hutchins DA, Huang B, Wang L, Zheng Y, Jin P, Cai X, Häder DP, Li W, Xu K, Liu N, Riebesell U. 2012. Rising CO_2 and increased light exposure synergistically reduce marine primary productivity. *Nature Climate Change* **2**:519–523 DOI 10.1038/nclimate1507.

Gao G, Zhong Z, Zhou X, Xu J. 2016b. Changes in morphological plasticity of Ulva prolifera under different environmental conditions: a laboratory experiment. *Harmful Algae* **59**:51–58 DOI 10.1016/j.hal.2016.09.004.

García-Sánchez MJ, Fernández JA, Niell X. 1994. Effect of inorganic carbon supply on the photosynthetic physiology of *Gracilaria tenuistipitata*. *Planta* **194**:55–61 DOI 10.1007/bf00201034.

Gattuso JP, Magnan A, Billé R, Cheung WW, Howes EL, Joos F. 2015. Contrasting futures for ocean and society from different anthropogenic CO_2 emissions scenarios. *Science* **349**:45–55 DOI 10.1126/science.aac4722.

Giordano M, Beardall J, Raven JA. 2005. CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. *Annual Review Plant Biology* **56**:99–131 DOI 10.1146/annurev.arplant.56.032604.144052.

Green LA, Neefus CD. 2016. Effects of temperature, light level, and photoperiod on the physiology of Porphyra umbilicalis Kützing from the Northwest Atlantic, a candidate for aquaculture. *Journal of Applied Phycology* **28(3)**:1815–1826 DOI 10.1007/s10811-015-0702-6.

Gutow L, Rahman MM, Bartl K, Saborowski R, Bartsch I, Wiencke C. 2014. Ocean acidification affects growth but not nutritional quality of the seaweed *Fucus vesiculosus* (Phaeophyceae, Fucales). *Journal of Experimental Marine Biology and Ecology* **453**:84–90 DOI 10.1016/j.jembe.2014.01.005.

Heinrich S, Valentin K, Frickenhaus S, John U, Wiencke C. 2012. Transcriptomic analysis of acclimation to temperature and light stress in Saccharina latissima (Phaeophyceae). *PLOS ONE* **7(8)**:e44342 DOI 10.1371/journal.pone.0044342.

Jin P, Wang T, Liu N, Dupont S, Beardall J, Boyd PW, Riebesell U, Gao K. 2015. Ocean acidification increases the accumulation of toxic phenolic compounds across trophic levels. *Nature Communications* **6**:8714 DOI 10.1038/ncomms9714.

Johnson MD, Price NN, Smith JE. 2014. Contrasting effects of ocean acidification on tropical fleshy and calcareous algae. *PeerJ* **2**:e411 DOI 10.7717/peerj.411.

Kang EJ, Kim JH, Kim K, Choi HG, Kim KY. 2014. Re-evaluation of green tide-forming species in the Yellow Sea. *Algae* **29(4)**:49–59 DOI 10.4490/algae.2014.29.4.267.

Kendirlioglu G, Agirman N, Cetin A. 2015. The effects of photoperiod on the growth, protein amount and pigment content of *Chlorella vulgaris*. *Turkish Journal of Science & Technology* **10(2)**:7–10.

Koch M, Bowes G, Ross C, Zhang XH. 2013. Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Global Change Biology* **19(1)**:103–132 DOI 10.1111/j.1365-2486.2012.02791.x.

Kroeker KJ, Kordas RL, Crim R, Hendriks IE, Ramajo L, Singh GS. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biology* **19(6)**:1884–1896 DOI 10.1111/gcb.12179.

Kübler JE, Johnston AM, Raven JA. 1999. The effects of reduced and elevated CO_2 and O_2 on the seaweed Lomentaria articulata. *Plant, Cell & Environment* **22(10)**:1303–1310 DOI 10.1046/j.1365-3040.1999.00492.x.

Li Y, Zhong J, Zheng M, Zhuo P, Xu N. 2018. Photoperiod mediates the effects of elevated CO_2 on the growth and physiological performance in the green tide alga *Ulva prolifera*. *Marine Environmental Research* **141**:24–29 DOI 10.1016/j.marenvres.2018.07.015.

Ma J, Ji J, Xu R, He P, Zhang T, Wang X, Li Y, Ren S, Xu P, Lu Q. 2009. Preliminary study on life history of *Ulva linza* Linnaeus [Enteromorpha linza (L.) J. Ag.]. *Journal of Fisheries of China* **33**:45–52.

Maberly SC, Raven JA, Johnston AM. 1992. Discrimination between ^{12}C and ^{13}C by marine plants. *Oecologia* **91(4)**:481–492 DOI 10.1007/bf00650320.

Mercado JM, Javier F, Gordillo L, Niell FX, Figueroa FL. 1999. Effects of different levels of CO_2 on photosynthesis and cell components of the red alga Porphyra leucosticta. *Journal of Applied Phycology* **11(5)**:455–461 DOI 10.1023/A:1008194223558.

Nielsen MV. 1997. Growth, dark respiration and photosynthetic parameters of the coccolithophorid Emiliania Huxleyi (Prymnesiophyceae) acclimated to different day length-irradiance combinations1. *Journal of Phycology* **33(5)**:818–822 DOI 10.1111/j.0022-3646.1997.00818.x.

Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437**:681–686 DOI 10.1038/nature04095.

Pierrot D, Lewis E, Wallace DWR. 2006. MS Excel program developed for CO_2 system calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee.

Porra RJ, Thompson WA, Kriedemann PE. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted

with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* **975(3)**:384–394 DOI 10.1016/S0005-2728(89)80347-0.

Rautenberger R, Fernandez PA, Strittmatter M, Heesch S, Cornwall CE, Hurd CL, Roleda MY. 2015. Saturating light and not increased carbon dioxide under ocean acidification drives photosynthesis and growth in *Ulva rigida* (Chlorophyta). *Ecology and Evolution* **5(4)**:874–888 DOI 10.1002/ece3.1382.

Raven JA. 2003. Inorganic carbon concentrating mechanisms in relation to the biology of algae. *Photosynthesis Research* **77(2–3)**:155–171 DOI 10.1023/A:1025877902752.

Raven JA, Ball LA, Beardall J, Giordano M, Maberly SC. 2005. Algae lacking carbon-concentrating mechanisms. *Canadian Journal of Botany* **83(7)**:879–890 DOI 10.1139/b05-074.

Raven JA, Beardall J, Sánchez-Baracaldo P. 2017. The possible evolution and future of CO_2-concentrating mechanisms. *Journal of Experimental Botany* **68(14)**:3701–3716 DOI 10.1093/jxb/erx110.

Reidenbach LB, Fernandez PA, Leal PP, Noisette F, McGraw CM, Revill AT. 2017. Growth, ammonium metabolism, and photosynthetic properties of *Ulva australis* (Chlorophyta) under decreasing pH and ammonium enrichment. *PLOS ONE* **12(11)**:e0188389 DOI 10.1371/journal.pone.0188389.

Rost B, Riebesell U, Burkhardt S, Sültemeyer D. 2003. Carbon acquisition of bloom-forming marine phytoplankton. *Limnology and Oceanography* **48(1)**:55–67 DOI 10.4319/lo.2003.48.1.0055.

Rost B, Riebesell U, Sültemeyer D. 2006. Carbon acquisition of marine phytoplankton: effect of photoperiod length. *Limnology and Oceanography* **51(1)**:12–20 DOI 10.4319/lo.2006.51.1.0012.

Roth-Schulze AJ, Thomas T, Steinberg P, Deveney MR, Tanner JE, Wiltshire KH. 2018. The effects of warming and ocean acidification on growth, photosynthesis, and bacterial communities for the marine invasive macroalga *Caulerpa taxifolia*. *Limnology and Oceanography* **63(1)**:459–471 DOI 10.1002/lno.10739.

Roy RN, Roy LN, Vogel KM, Porter-Moore C, Pearson T, Good CE, Millero FJ, Campbell DM. 1993. The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45 C. *Marine Chemistry* **44(2–4)**:249–267 DOI 10.1016/0304-4203(93)90207-5.

Sabine CL, Feely RA, Gruber N, Key RM, Lee K, Bullister JL. 2004. The oceanic sink for anthropogenic CO_2. *Science* **305(5682)**:367–371 DOI 10.1126/science.1097403.

Schreiber U. 2004. Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: an overview. *Chlorophyll a Fluorescence* **19**:279–319 DOI 10.1007/978-1-4020-3218-9_11.

Singh SP, Singh P. 2015. Effect of temperature and light on the growth of algae species: a review. *Renewable and Sustainable Energy Reviews* **50**:431–444 DOI 10.1016/j.rser.2015.05.024.

Smith SV. 1981. Marine macrophytes as a global carbon sink. *Science* **211(4484)**:838–840 DOI 10.1126/science.211.4484.838.

Wittmann AC, Pörtner HO. 2013. Sensitivities of extant animal taxa to ocean acidification. *Nature Climate Change* **3**:995–1001 DOI 10.1038/nclimate1982.

Wu Y, Gao K, Riebesell U. 2010. CO_2-induced seawater acidification affects physiological performance of the marine diatom Phaeodactylum tricornutum. *Biogeosciences* **7**:2915–2923 DOI 10.5194/bg-7-2915-2010.

Xu J, Gao K. 2012. Future CO_2-induced ocean acidification mediates the physiological performance of a green tide alga. *Plant Physiology* **160**:1762–1769 DOI 10.1104/pp.112.206961.

Xu J, Gao K. 2015. Photosynthetic performance of the red alga *Pyropia haitanensis* during emersion, with special reference to effects of solar UV radiation, dehydration and elevated CO_2 concentration. *Photochemistry and Photobiology* **91(6)**:1376–1381 DOI 10.1111/php.12531.

Xu Q, Zhang H, Cheng Y. 2016. Multi-sensor monitoring of *Ulva prolifera* blooms in the Yellow Sea using different methods. *Frontiers of Earth Science* **10(2)**:378–388 DOI 10.1007/s11707-015-0528-1.

Xu Z, Gao G, Xu J, Wu H. 2017. Physiological response of a golden tide alga (*Sargassum muticum*) to the interaction of ocean acidification and phosphorus enrichment. *Biogeosciences* **4**:671–681 DOI 10.5194/bg-14-671-2017.

Young CS, Gobler CJ. 2016. Ocean acidification accelerates the growth of two bloom-forming macroalgae. *PLOS ONE* **11(5)**:e0155152 DOI 10.1371/journal.pone.0155152.

Zhang D, Zhang Q, Yang X. 2017. Seasonal dynamics of photosynthetic activity in the representative brown macroalgae Sagrassum thunbergii (Sargassaceae Phaeophyta). *Plant Physiology and Biochemistry* **120**:88–94 DOI 10.1016/j.plaphy.2017.09.025.

Zou D. 2005. Effects of elevated atmospheric CO_2 on growth, photosynthesis and nitrogen metabolism in the economic brown seaweed, *Hizikia fusiforme* (Sargassaceae, Phaeophyta). *Aquaculture* **250(3–4)**:726–735 DOI 10.1016/j.aquaculture.2005.05.014.

Zucchi MR, Necchi O. 2001. Effects of temperature, irradiance and photoperiod on growth and pigment content in some freshwater red algae in culture. *Phycological Research* **49(2)**:103–114 DOI 10.1111/j.1440-1835.2001.tb00240.x.