

## Research Article

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# Effects of future climate conditions on photosynthesis and biochemical component of *Ulva pertusa* (Chlorophyta)

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*Ulva pertusa*, a common bloom-forming green alga, was used as a model system to examine the effects of elevated carbon dioxide (CO<sub>2</sub>) and temperature on growth and photosynthetic performance. To do this, *U. pertusa* was grown under four temperature and CO<sub>2</sub> conditions; ambient CO<sub>2</sub> (400 μatm) and temperature (16°C) (i.e., present), elevated temperature only (19°C) (ET; i.e., warming), elevated CO<sub>2</sub> only (1,000 μatm) (EC; i.e., acidification), and elevated temperature and CO<sub>2</sub> (ET and EC; i.e., greenhouse), and its steady state photosynthetic performance evaluated. Maximum gross photosynthetic rates (GP<sub>max</sub>) were highest under EC conditions and lowest under ET conditions. Further, ET conditions resulted in decreased rate of dark respiration (R<sub>d</sub>), but growth of *U. pertusa* was higher under ET conditions than under ambient temperature conditions. In order to evaluate external carbonic anhydrase (eCA) activity, photosynthesis was measured at 70 μmol photons m<sup>-2</sup> s<sup>-1</sup> in the presence or absence of the eCA inhibitor acetazolamide (AZ), which inhibited photosynthetic rates in all treatments, indicating eCA activity. However, while AZ reduced *U. pertusa* photosynthesis in all treatments, this reduction was lower under ambient CO<sub>2</sub> conditions (both present and warming) compared to EC conditions (both acidification and greenhouse). Moreover, Chlorophyll *a* and glucose contents in *U. pertusa* tissues declined under ET conditions (both warming and greenhouse) in conjunction with reduced GP<sub>max</sub> and R<sub>d</sub>. Overall, our results indicate that the interaction of EC and ET would offset each other's impacts on photosynthesis and biochemical composition as related to carbon balance of *U. pertusa*.

**Key Words:** acidification; CO<sub>2</sub>; greenhouse; photosynthesis; temperature; *Ulva pertusa*; warming

## INTRODUCTION

Atmospheric concentrations of carbon dioxide (CO<sub>2</sub>) have rapidly increased since the time of the Industrial Revolution, leading to changes in the chemical composition of seawater. The AIFI scenario of the Intergovernmental Panel on Climate Change (IPCC) projects that the atmospheric CO<sub>2</sub> concentration will have increased to 970 μatm nearly three times the present concentration by the end of the current century (IPCC 2007). Concomitantly, the average pH of seawater is expected to drop by ap-

proximately 0.46 units from current levels, with both CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> concentrations increasing and CO<sub>3</sub><sup>2-</sup> decreasing. These changes caused by increasing atmospheric CO<sub>2</sub> will also be associated with increased ocean temperature and acidity, sea-level rise, and more frequent natural disasters (IPCC 2007, Doney et al. 2009). There is general agreement that these impacts will be some of the most notable environmental changes in the ocean during the coming decades (e.g., Hansen et al. 2005).



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There are several potential biological implications of the expected environmental changes associated with ocean acidification. For example, current CO<sub>2</sub> concentrations, in the form of dissolved inorganic carbon (DIC) in seawater, are not high enough to saturate photosynthesis of marine primary producers, and therefore, photosynthesis in macroalgae and phytoplankton could be saturated under current CO<sub>2</sub> conditions by using carbon concentration mechanisms (CCMs) (Raven 1997), such as those associated with the enzyme carbonic anhydrase (CA), which catalyzes dehydration of HCO<sub>3</sub><sup>-</sup> to CO<sub>2</sub>. However, the exact mechanisms by which this occurs may differ at phylogenetic levels, from ecotypes to phyla (Johnston et al. 1992, Mercado et al. 1997, Brading et al. 2011). Indeed, most macroalgae involve CCMs and thus use HCO<sub>3</sub><sup>-</sup> for photosynthesis; however, photosynthesis does not generally appear saturated under present DIC conditions (Koch et al. 2013). From previous research, several species of *Ulva* have shown variation in acclimations to changes in DIC, such as the use of HCO<sub>3</sub><sup>-</sup>, the extent of carbonic anhydrase activity, and DIC saturation states. Indeed, many researchers predicted that the change in ocean CO<sub>2</sub> concentrations could directly affect CCM activities in many species because increased CO<sub>2</sub> enhances the dependence of carbon by diffusion. In addition, the down-regulation of CCMs appears through decreased HCO<sub>3</sub><sup>-</sup> usage and increased reliance on CO<sub>2</sub> utilization, and mechanism that has been supported by many laboratory and *in situ* experiments (Gao et al. 2012).

Some metabolic changes including photosynthesis, respiration, and enzyme activity occur as a result of increased temperature (Atkin et al. 2000, Atkin and Tjoelker 2003, Vona et al. 2004), which in turn, alters the physiological responses of the macroalgae to environmental changes (Kübler and Davison 1995, Zou and Gao 2013). Simultaneously, increased atmospheric CO<sub>2</sub> will result in ocean acidification and many unpredictable effects (Doney et al. 2009). Considerable research was carried out on the impacts of these factors; however, most has focused primarily on the processes of biological calcification, the general impacts on phytoplankton communities, and only a few have focused on selected macroalgae. Further, few of these studies have considered the combined impacts of these environmental changes on a bloom-forming green alga using ecologically relevant conditions (e.g., Xu and Gao 2012). Here, we consider the impact of these factors on *U. pertusa* using environmentally relevant levels of CO<sub>2</sub> and temperature.

*Ulva* species, the globally important bloom-forming green algae, grow in a wide range of temperatures, rang-

ing from 10-30°C, with the highest growth rates generally occurring at 15-20°C (Taylor et al. 2001). Likewise, the sporelings of *U. intestinalis* (*Enteromorpha intestinalis*) grow in a wide range of temperatures, with the highest growth rates occurring at 15 and 20°C (Kim and Lee 1996). Further, the floating *U. linza*, collected in the early summer from the Yellow Sea, exhibits exponential growth at 10-15°C, and tissue senescence occurs at temperatures over 20°C, but gross photosynthesis (GP) is higher at 20-25°C (Kim et al. 2011, Kang et al. 2016). In *U. rigida* higher growth occurs below 17°C, whereas growth declines above 17°C (de Casabianca et al. 2002). *U. pertusa* from eelgrass beds in Korea exhibit high growth rates at temperatures between 10 and 25°C (Choi 2003). Together, these different growth rates reflect the temperature regimes these algae experience in natural habitats, and reflect the seasonality and distribution of each species (Gessner 1970, Innes 1988, Lüning 1990).

Considerable research has demonstrated that photosynthetic rates of marine plants are enhanced by increased CO<sub>2</sub> concentrations. However, photosynthetic activity depends not only on CO<sub>2</sub> concentration but also on nutrient levels, light and temperature conditions (Zimmerman et al. 1997, Gordillo et al. 2001, 2003, Fu et al. 2007, Zou et al. 2011). For example, the marine picocyanobacteria *Synechococcus* and *Prochlorococcus* were examined under four conditions that combined changes in CO<sub>2</sub> concentration and temperature (Fu et al. 2007). Growth and photosynthesis of *Prochlorococcus* were unaffected by increasing CO<sub>2</sub> and temperature, whereas growth and photosynthesis in *Synechococcus* were stimulated by increasing temperature, but showed synergistic responses with increasing CO<sub>2</sub> and temperature. The recruitment of algal turfs (mainly *Feldmannia* spp.) also has shown synergistic responses when exposed to combined future CO<sub>2</sub> and temperature conditions, with biomass increased two-fold relative to when the turfs were exposed to the single effects of either CO<sub>2</sub> or temperature alone (Connell and Russell 2010). Further, the effective quantum yield of algal turfs also increases when exposed to future CO<sub>2</sub> conditions, but decreases when exposed to elevated temperature alone. Olabarria et al. (2013) observed that both individuals and assemblages of rockpool macroalgae exhibit various responses to increased CO<sub>2</sub> and high temperature. Specifically, increased CO<sub>2</sub> and temperature resulted in decreases in macroalgae assemblage biomass because of changes in productivity and respiration. However, these experiments have not previously been conducted with a green tide forming eukaryotic alga where the impacts on natural communities may

be extreme.

*Ulva pertusa* (Chlorophyta) is a green tide species with outbreaks occurring in many coastal areas of Korea (Kim et al. 2004). Blooms of *U. pertusa*, are associated with elevated nutrients, especially nitrogen and phosphorous, and partially regulated by temperature, light and biotic factors (Valiela et al. 1997, Giannotti and McGlathery 2001). Despite the extensive understanding of the factors associated with green tide outbreaks, there are a few studies that allow us to predict the potential impacts of elevated atmospheric CO<sub>2</sub> levels and consequent climate change on these blooms. In this study we simultaneously evaluate the impacts of both elevated CO<sub>2</sub> and temperature on the growth of *U. pertusa*. We focus on biochemical composition, respiration, photosynthetic responses and growth rates as adaptations of *U. pertusa* to realistic future climate conditions.

## MATERIALS AND METHODS

### Sample collection and incubation

*U. pertusa* was collected in December 2010 from the intertidal zone at Wando (34°19'30" N, 126°49'50" E), on the southern coast of Korea. *Ulva* species, especially *U. pertusa* and *U. linza* are predominant in this area during winter. The average water temperature and salinity were 10°C and S = 32, respectively at the time of sampling. The samples were rinsed in filtered seawater to remove macroscopic epiphytes and were maintained in filtered, aerated seawater supplemented with F/2 nutrients (FRITZ Industries Inc., Greenville, TX, USA). Thalli were initially maintained at conditions of 16°C, S = 32, and 70 μmol photons m<sup>-2</sup> s<sup>-1</sup>, and a 12 : 12 h light : dark cycle for 3 days prior to experimental initiation.

### Experimental design

Four treatments were used to measure the individual and combined effects of CO<sub>2</sub> and temperature: ambient CO<sub>2</sub> (400 μatm) and temperature (16°C) (i.e., present); ambient CO<sub>2</sub> and elevated temperature (19°C) (ET; i.e., warming); elevated CO<sub>2</sub> (1,000 μatm) and ambient temperature (EC; i.e., acidification); and both elevated temperature and CO<sub>2</sub> (ET and EC combined; i.e., greenhouse). Different CO<sub>2</sub> concentrations were provided by mixing air with the appropriate amounts of CO<sub>2</sub> obtained from a compressed CO<sub>2</sub> cylinder, as described by Kim et al. (2008). Carbon dioxide (CO<sub>2</sub>) concentrations were

monitored in the resulting air-CO<sub>2</sub> mixtures using a CO<sub>2</sub> analyzer (LI-840A; LI-COR, Lincoln, NE, USA). These mixtures were bubbled through the media, and CO<sub>2</sub> was monitored daily via measurements of the pH of culture media using a pH-meter (Meterlab PHM210; Radiometer Analytical SAS, Lyon, France). The maximum variation of CO<sub>2</sub> in individual cultures was 5%, and CO<sub>2</sub> inputs were adjusted daily in order to maintain constant levels in each experimental treatment.

Total seawater alkalinity (TA) and total DIC were measured with a potentiometric titration system (765 Dosimat; Metrohm AG, Herisau, Switzerland), combined with a ROSS half-cell pH electrode (Orion 8101BNWP; Thermo Scientific, Waltham, MA, USA) and a sure-flow reference electrode (Orion 900200; Thermo Scientific). Proportions of the carbon species in the seawater were calculated from the TA and DIC values using CO2SYS software (Lewis and Wallace 1998). The TA and DIC measurements were checked for accuracy against certified reference materials (distributed by A. Dickson, Scripps Institution of Oceanography) used here as standards. The precisions of the measurement were approximately ±2 μmol kg<sup>-1</sup> for TA and ±1.5 μmol kg<sup>-1</sup> for DIC. Temperature and irradiance were also monitored using a temperature/light data logger (UA-002-64; Onset, Pocasset, MA, USA) calibrated with a thermometer during the experiment. The experimental temperatures were controlled using aquarium heaters (EHEIM Jager, Deizisau, Germany). Temperatures were maintained to within 0.1°C of the targeted temperature throughout the experiment.

### Photosynthetic rates

Photosynthetic rates were measured using an oxygen closed-chamber method consisting of a 2-mm oxygen dipping probe (DP-PSt3) connected to Fibox3 (PreSens, Regenburb, Germany) at eight irradiances (0, 10, 45, 80, 150, 245, and 450 μmol photons m<sup>-2</sup> s<sup>-1</sup>) derived from a halogen lamp (KL2500; SCHOTT, Mainz, Germany). Temperature and CO<sub>2</sub> concentrations were maintained at each target level during the photosynthetic measurement. The photosynthesis-irradiance relationships for distinction of photosynthetic traits were fitted to a non-linear mathematical function that represented the double exponential function (Platt et al. 1980).

### External CA activities

In order to assess the external carbonic anhydrase (eCA) activity, photosynthesis was measured with and

without acetazolamide (AZ) under 70  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . AZ inhibits photosynthesis using  $\text{HCO}_3^-$ , hence the results show  $\text{CO}_2$  utilized during photosynthesis when AZ was added to the seawater media. AZ was prepared in 0.5 N NaOH and added to the medium at a concentration of 60  $\mu\text{M}$ . Experiments with AZ used the same irradiance levels as were used in the incubation conditions.

### Chlorophyll *a* (Chl *a*) fluorescence

Chl *a* fluorescence was measured using Diving-PAM (Walz GmbH, Effeltrich, Germany). Effective quantum yield ( $\Phi_{\text{PSII}}$ ) was calculated as:

$$\Phi_{\text{PSII}} = (F_m' - F) / F_m'$$

, where  $F$  and  $F_m'$  represent the steady-state fluorescence and maximum fluorescence measured in the light, respectively. Rapid light curves (RLC) were determined according to Ralph and Gademann (2005). Relative electron transport rate ( $r\text{ETR}$ ) was calculated as:

$$r\text{ETR} = \Phi_{\text{PSII}} \times \text{irradiance}$$

, with two ambiguous factors (the partitioning of light energy by photosystem [PS] I and PSII, and the absorption factor) were not considered. Chl *a* fluorescence parameters from  $r\text{ETR}$ -irradiance curves were calculated using a nonlinear regression equation (Platt et al. 1980).

### Growth rates

*U. pertusa* thalli were prepared by slicing individual 20 mm diameter disks from near the base of each thallus using a cork borer. To reduce the effects of damage at the disk margins, disks were cut one day prior to the start of the growth experiment. The growth rate ( $\mu$ ) was calculated as:

$$\mu = \ln(A - A_0) / (T - T_0)$$

, where  $A$  and  $A_0$  represent the areas at time  $T$  (after 10 days) and  $T_0$  (the initial day), respectively.

### Chl *a*, tissue nutrient, and glucose contents

Chl *a* contents were determined using a spectrophotometer (Helios  $\alpha$  UV-Vis; Unicam, Cambridge, UK). Each *U. pertusa* disk that was used to evaluate growth above was extracted in 20 mL glass vials with 8 mL of *N,N*-di-

methylformamide at 4°C for 24 h in the dark. The Chl *a* content was calculated as:

$$\text{Chl } a \text{ (mg L}^{-1}\text{)} = 12.70A_{664.5} - 2.97A_{647}$$

, where  $A_{664.5}$  and  $A_{647}$  represent absorbance at 664.5 nm and 647 nm, respectively (Inskeep and Bloom 1985).

Carbon (C) and nitrogen (N) contents within the *U. pertusa* tissues were determined from individual disks using an elemental analyzer (EA 1110; CE Instruments, Milan, Italy). Samples were dried at 60°C for 24 h, and homogenized by mill grinding (Mixer Mill MM301; Retsch, Hann, Germany). Tissue phosphorous (P) was extracted using an alkaline persulfate digestion, and determined using a standard colorimetric phosphate protocol (Menzel and Corwin 1965).

Glucose content within the *U. pertusa* tissues was determined by using 3,5-dinitrosalicylic acid to measure reducing sugar (Wood and Bhat 1988); 70 mL of 0.1 M HCl was added to 3 g of dried sample in an Erlenmeyer flask, and reducing sugar was extracted by autoclaving at 121°C and 1.5 atm for 15 min.

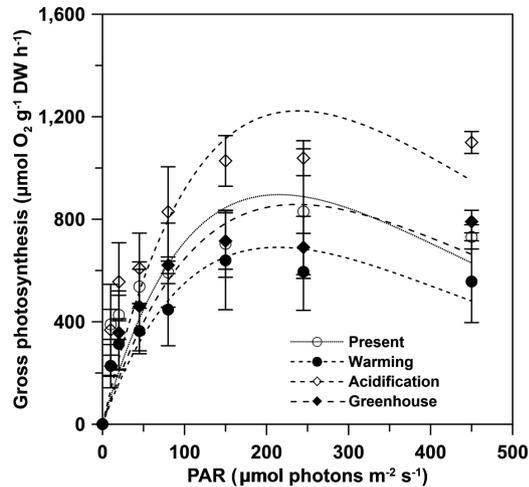
### Statistical analysis

All statistical analyses were performed using the SPSS 20.0 statistical software. Data normality and homogeneity of variance were determined using the Kolmogorov-Smirnov normality test and Levene's homogeneity of variance test, respectively. One-way analysis of variance (ANOVA) and two-way ANOVAs were used to compare the effects of  $\text{CO}_2$  concentrations and temperatures on photosynthetic parameters, growth rate, Chl *a* concentration, and reducing sugar. Nonparametric tests (Kruskal-Wallis test) were conducted for data that were not normally distributed. When significant differences were detected, specific differences between treatment pairs were determined using Tukey's multiple comparisons. A paired-sample t-test was used to aid in differentiating the eCA activity before and after the addition of eCA inhibitor.

## RESULTS

### Photosynthesis and Chl *a* fluorescence

Gross photosynthesis (GP) in *U. pertusa* determined from the photosynthesis-irradiance (P-E) curves did not vary significantly among the four  $\text{CO}_2$  and temperature treatments. Further, GP was saturated at 100-150  $\mu\text{mol}$



**Fig. 1.** Photosynthesis versus irradiance curves of *Ulva pertusa* under four CO<sub>2</sub> and temperature conditions. PAR, photosynthetic active radiation, i.e., irradiance. Data are presented as mean  $\pm$  standard deviation ( $n = 3$ ).

photons  $m^{-2} s^{-1}$  and either decreased or reached a plateau over 150  $\mu mol photons m^{-2} s^{-1}$  in all treatments (Fig. 1). However, GP in the acidification treatment showed distinctly higher photosynthesis and was 1.2-2 fold higher when the alga was exposed to over 150  $\mu mol photons m^{-2} s^{-1}$  under all measured irradiances. The photosynthetic parameters obtained from the P-E curves are shown in Table 1. Maximum gross photosynthetic rates ( $GP_{max}$ ) were greatest ( $1,046.9 \pm 66.5 \mu mol O_2 g^{-1} DW h^{-1}$ ) under acidification conditions, and lowest ( $622.9 \pm 133.7 \mu mol$

$O_2 g^{-1} DW h^{-1}$ ) under warming conditions. There was a trend towards higher rates of photosynthesis in the elevated CO<sub>2</sub> conditions (acidification and greenhouse; hereafter EC) ( $F = 6.986, p = 0.030$ ), while the elevated temperature condition (warming and greenhouse; hereafter ET) negatively impacted  $GP_{max}$  ( $F = 6.366, p = 0.036$ ). Given that increased photosynthesis under EC was offset by reduced photosynthetic rates under ET, GP of *U. pertusa* grown under greenhouse conditions did not exhibit any differences in GP relative to present conditions. There were also no differences in photosynthetic efficiency ( $\alpha$ ) and minimum saturation irradiance ( $E_k$ ) among the treatments. Dark respiration ( $R_d$ ) ranged between 68.6 and 252.7  $\mu mol O_2 g^{-1} DW h^{-1}$ , with the highest  $R_d$ , observed under the present conditions nearly 2.5-fold higher than under the warming conditions. Consequently,  $R_d$  appeared to depend most upon temperature change, i.e.,  $R_d$  of ambient temperature had twice as high as ET ( $F = 11.206, p = 0.010$ ), while  $R_d$  did not appear to be impacted by EC conditions ( $p > 0.05$ ).

The maximum quantum yields of PSII ( $F_v / F_m$ ) ranged from 0.74 to 0.77, but there were no significant difference among the treatments (Table 2). However, there was a trend in which  $F_v / F_m$  was slightly decreased under ET conditions and increased under EC conditions, but again these patterns were no significant. Similarly, maximum electron transport rates ( $rETR_{m,RLC}$ ), electron transport efficiency ( $\alpha_{RLC}$ ) and minimum saturation irradiance ( $E_{k,RLC}$ ), as determined from the RLC, were also not significantly different among the treatments.

**Table 1.** Photosynthetic parameters of *Ulva pertusa* obtained from the four temperature and CO<sub>2</sub> conditions ( $n = 3$ , mean  $\pm$  SD)

	$GP_{max}$	$\alpha$	$E_k$	$R_d$
Present	$783.7 \pm 232.4^{ab}$	$31.5 \pm 22.3$	$40 \pm 37$	$228.4 \pm 11.1^a$
Warming	$622.9 \pm 133.7^a$	$12.9 \pm 6.3$	$53 \pm 17$	$91.7 \pm 20.6^b$
Acidification	$1,046.9 \pm 66.5^b$	$34.4 \pm 25.2$	$42 \pm 23$	$202.4 \pm 62.8^{ab}$
Greenhouse	$793.5 \pm 67.1^{ab}$	$19.5 \pm 16.8$	$62 \pm 38$	$149.5 \pm 71.6^{ab}$

Different superscripted letters indicate significant differences based on Tukey's multiple comparison ( $p < 0.05$ ).

SD, standard deviation;  $GP_{max}$  and  $R_d$ , maximum gross photosynthetic rate and dark respiration rate ( $\mu mol O_2 g^{-1} DW h^{-1}$ ),  $\alpha$ , photosynthetic efficiency ( $\mu mol O_2 g^{-1} DW h^{-1} [\mu mol photons m^{-2} s^{-1}]^{-1}$ ),  $E_k$ , saturation irradiance ( $\mu mol photons m^{-2} s^{-1}$ ).

**Table 2.** Chlorophyll *a* fluorescence parameters of *Ulva pertusa* obtained from the four temperature and CO<sub>2</sub> conditions ( $n = 3$ , mean  $\pm$  SD)

	$F_v / F_m$	$rETR_{m,RLC}$	$\alpha_{RLC}$	$E_{k,RLC}$
Present	$0.765 \pm 0.021$	$204 \pm 32$	$0.939 \pm 0.013$	$217 \pm 34$
Warming	$0.741 \pm 0.040$	$165 \pm 9$	$0.837 \pm 0.114$	$198 \pm 23$
Acidification	$0.768 \pm 0.022$	$166 \pm 20$	$0.924 \pm 0.030$	$180 \pm 27$
Greenhouse	$0.769 \pm 0.019$	$192 \pm 10$	$0.943 \pm 0.011$	$204 \pm 10$

Different superscripted letters indicate significant differences based on Tukey's multiple comparison ( $p < 0.05$ ).

SD, standard deviation;  $F_v / F_m$ , maximum quantum yield;  $rETR_{m,RLC}$ , maximum relative electron transport rate ( $\mu mol e^{-1} m^{-2} s^{-1}$ );  $\alpha_{RLC}$ , electron transport efficiency ( $\mu mol e^{-1} m^{-2} s^{-1} [\mu mol photons m^{-2} s^{-1}]^{-1}$ );  $E_{k,RLC}$ , saturation light of the rapid light curves (RLC) ( $\mu mol photons m^{-2} s^{-1}$ ).

### External CA activity

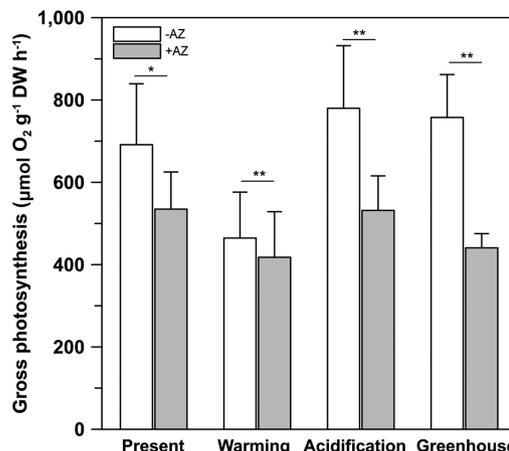
Gross photosynthesis (GP) in *U. pertusa* measured in the absence of AZ ranged from 386.5 to 876.3  $\mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$  when held at 70  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  (Fig. 2). Indeed, GP was significantly inhibited (by 8-52%) in the presence of AZ ( $t = 5.180, p < 0.001$ ). While *U. pertusa* possesses eCA activity as one of CCMs, this activity did not differ among the treatments. However, there was significant reduction (by 50%) in GP under the ambient  $\text{CO}_2$  conditions (present and warming) relative to EC conditions ( $F = 9.825, p = 0.017$ ). Further, there were no significant impacts to photosynthesis caused by adding AZ under the different temperatures ( $p > 0.05$ ), and not there were impacts of the combined effects of  $\text{CO}_2$  and temperature on eCA activity compared to the present condition.

### Chl *a*, tissue nutrient, and glucose contents

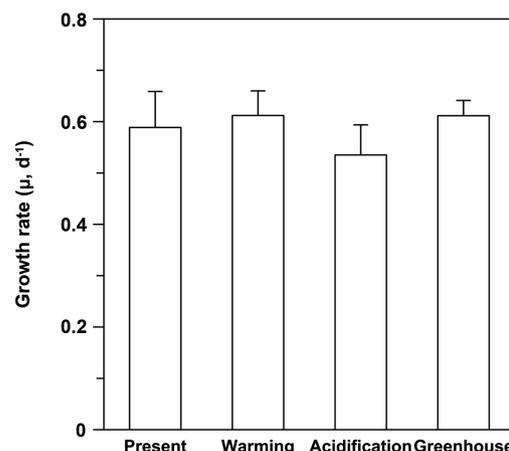
The trend for Chl *a* contents was similar to that for photosynthetic rate (Table 3). Specifically, there was no significant difference among the treatments, though a trend was observed in which, Chl *a* content was higher under EC conditions than under ambient  $\text{CO}_2$  conditions, but these differences were reduced with increasing temperature. Together, this indicates that Chl *a* contents in *U. pertusa* tissues were not different under greenhouse condition relative to present condition, suggesting that ET offsets the increased Chl *a* concentration that is induced by EC.

The carbon and nitrogen contents in *U. pertusa* tissues were lower under ET conditions than in any of the other treatments (Table 3). Tissue P, in contrast, was highest under EC conditions and lowest under present conditions. However, individually and in combination, EC or ET levels significantly impacted neither elemental tissue contents or their ratios.

Glucose content within *U. pertusa* tissues was dependent on temperature ( $F = 66.589, p < 0.001$ ) but not  $\text{CO}_2$  conditions. Specifically, glucose was significantly greater



**Fig. 2.** Photosynthesis of *Ulva pertusa* under four  $\text{CO}_2$  and temperature conditions. Filled bars represent photosynthesis when acetazolamide (AZ) added. Data are presented as mean  $\pm$  standard deviation ( $n = 3, *p < 0.05, **p < 0.01$  for paired t-test between -AZ and +AZ and  $p > 0.05$  for photosynthesis between the four conditions).



**Fig. 3.** Growth rate ( $\mu$ ) of *Ulva pertusa* under four  $\text{CO}_2$  and temperature conditions. Data are presented as mean  $\pm$  standard deviation ( $n = 5, p > 0.05$ ).

under EC conditions than present  $\text{CO}_2$  concentrations ( $F = 5.328, p = 0.05$ ), while ET induced a significant decrease in the glucose content, by approximately 12% (Table 3).

**Table 3.** Chlorophyll *a* concentration ( $\text{mg Chl } a \text{ g}^{-1} \text{ FW}$ ), tissue elemental contents (%) and glucose content ( $\text{mg g}^{-1} \text{ DW}$ ) of *Ulva pertusa* obtained from the four temperature and  $\text{CO}_2$  conditions ( $n = 3-5, \text{mean} \pm \text{SD}$ )

	Chl <i>a</i>	C	N	P	Reducing sugar
Present	1.698 $\pm$ 0.309	36.44 $\pm$ 0.50	4.57 $\pm$ 0.15	0.906 $\pm$ 0.096	125 $\pm$ 3 <sup>a</sup>
Warming	1.575 $\pm$ 0.291	35.22 $\pm$ 0.92	4.52 $\pm$ 0.02	0.929 $\pm$ 0.153	109 $\pm$ 2 <sup>b</sup>
Acidification	1.969 $\pm$ 0.254	36.33 $\pm$ 0.49	4.58 $\pm$ 0.00	1.071 $\pm$ 0.079	131 $\pm$ 5 <sup>a</sup>
Greenhouse	1.773 $\pm$ 0.357	36.64 $\pm$ 0.09	4.64 $\pm$ 0.09	0.926 $\pm$ 0.041	113 $\pm$ 4 <sup>b</sup>

Different superscripted letters indicate significant differences based on Tukey's multiple comparison ( $p < 0.05$ ). SD, standard deviation.

## Growth rate

Growth rates ( $\mu$ ) ranged from 0.493 to 0.681 day<sup>-1</sup>, with the lowest  $\mu$  occurring under EC conditions (Fig. 3). Although there were no significant differences among treatments, the  $\mu$  trended higher under ET conditions, irrespective of CO<sub>2</sub> levels ( $F = 4.319$ ,  $p = 0.054$ ), and it appeared that EC did not impact the growth rates of *U. pertusa* ( $p > 0.1$ ). Lastly, the combined effect of CO<sub>2</sub> and temperature did not elicit any difference on the growth rates.

## DISCUSSION

It was shown previously that the growth rates of *Ulva* spp. were not significantly affected by enriched CO<sub>2</sub> concentrations (Israel and Hophy 2002). The growth rate of *U. pertusa* in this study also did not differ between ambient and enhanced CO<sub>2</sub> conditions, despite a slight upward trend in photosynthetic rates as CO<sub>2</sub> levels rose (Figs 1 & 3). The growth of *U. pertusa* appears to be largely decoupled from photosynthesis; for example, photosynthetic rates were low under ET conditions, whereas the species growth rates were slightly higher under ET conditions (Figs 2 & 3). This finding indicates that growth is regulated not only by photosynthesis but also by other mechanisms, including respiration rate and release of organic C compounds that restrict the contribution of C fixation to growth (Davison 1991). The C balance of photosynthesis and respiration controls plant growth (Cheng et al. 2000). The growth rates of *U. linza*, which with their massive green tide floating in the Yellow Sea in early summer, were close to zero at higher temperatures despite having high level of photosynthesis, most likely due to high respiration and extensive fragmentation offsetting increasing rates of photosynthesis (Kim et al. 2011, Kang et al. 2014, 2016). In our study, the rate of photosynthesis was not significantly different among the treatments, but respiration decreased as temperature increased, and trends in growth rates corresponded to photosynthesis / respiration ratios (GP / R<sub>d</sub> ratios) (data not shown). GP / R<sub>d</sub> ratios ranged from 2.5 to 4.9 in the four treatments, with these ratios being slightly higher under ET conditions, but not impacted by CO<sub>2</sub> concentration. Such high GP / R<sub>d</sub> ratios suggest that photosynthates are used less for respiration and more for growth (Zou and Gao 2014). Indeed, Gordillo et al. (2001) showed a doubling in growth of *U. rigida* at high CO<sub>2</sub> concentrations that were concurrent with a decline in organic carbon release when N was not limiting. Dissolved organic carbon (DOC) exudation, however, has

been shown to increase in some eukaryotic phytoplankton under EC (Engel et al. 2005). Thus, it is possible that *U. pertusa* releases a large amount of DOC under EC, and this may affect its growth rate.

The highest growth rates of *Ulva* spp. have been observed at temperature between 10 and 20°C (Taylor et al. 2001), with various optimal temperatures for photosynthesis of *U. pertusa* reported for different locations: 15°C (Floreto et al. 1993), 20–25°C (Murase et al. 1993), and 20°C in Korean coastal waters (Kim et al. 2004). These differences are based on physiological variations among ecotypes adapted to particular geographic and seasonal conditions (Young et al. 1987, Schaum et al. 2013). In rocky shores of the south coast of Korea, for example, an intense bloom of *U. pertusa* often occurs during spring when seawater temperatures range between 12 and 18°C (Kim et al. 2004). Our experiment assumed 16°C to be the ambient temperature, with 19°C the elevated temperature condition, which correspond to the optimal temperature range for growth of *Ulva* species reported by Taylor et al. (2001). Further, our results show that while photosynthesis is negatively affected by high temperatures, growth is not. In general, high temperatures led to increased rates of respiration (Atkin and Tjoelker 2003), although we found that R<sub>d</sub> was higher under ambient temperature conditions. The low R<sub>d</sub> of *U. pertusa* under ET conditions reduces the loss of fixed C during dark periods, thereby increasing the fixed C used for growth (Davison et al. 1991), which may explain why there were no differences in growth rates among the four treatments.

Low eCA activity shows that organisms prefer CO<sub>2</sub> or both HCO<sub>3</sub><sup>-</sup> and CO<sub>2</sub> as primary photosynthetic substrates (Mercado et al. 1997). *U. pertusa* exhibited significant eCA activity, but this activity did not change under EC and / or ET conditions (Fig. 2). This suggests that photosynthesis may be saturated under current oceanic DIC conditions and the species prefers to use CO<sub>2</sub>. This result is consistent with previous observations of CA activity in *Ulva* species (Björk et al. 1993), although some researchers have shown that AZ may significantly inhibit photosynthesis in *U. linza* (Israel and Hophy 2002).

In this study, increased CO<sub>2</sub> concentrations and temperatures did not enhance photosynthesis, growth rates or biochemical composition, with the exception of glucose content (Table 3). However, photosynthetic pigment composition did change with CO<sub>2</sub> concentration in previous studies. Specifically, *U. rigida* grown under different CO<sub>2</sub> conditions exhibits decreased pigment contents at high CO<sub>2</sub> (Gordillo et al. 2003), and the photosynthetic pigments of *Gracilaria tenuistipitata* likewise decreases

when DIC is enriched (5% CO<sub>2</sub>) (García-Sánchez et al. 1994). Further, Andria et al. (1999) showed similar results to those in our study in that DIC concentration did not impact the photosynthetic pigments of *Gracilaria* sp. In general, biochemical composition varies with nutrient conditions of the culture medium (Andria et al. 1999, Figueroa et al. 2009), and increasing CO<sub>2</sub> concentrations enhance photosynthetic activity and increase nutrient uptake (Webber et al. 1994). Plants use tissue nutrients to supply the nutrient demand for photosynthesis, thereby nutrient limitation may occur under CO<sub>2</sub> conditions. In this study, nutrients were replenished every 2 days, and as such nutrient limitation did not occur in any treatment, which may explain the absence of any significant differences in Chl *a* concentrations and / or tissue element contents. Glucose content, which is related to rates of photosynthesis and respiration, was lower under high as opposed to ambient temperatures. Carbohydrates storage is related to photosynthetic supply or respiratory demand (Falkowski and Raven 2007); given that, photosynthesis trended lower under ET condition than under present or EC conditions, possibly explaining why glucose production was reduced in this study. Simultaneously, respiration was also reduced, and this might be related to the need to maintain carbon balance at lower glucose levels.

It is often argued that global climate change causes more frequent harmful algal blooms (Dale et al. 2006) but our results suggest that this may not be the case with *U. pertusa*. Increased temperatures above typical temperatures in the spring when the bloom naturally occurs do not appear to increase *U. pertusa* growth rates. We suggest that *U. pertusa* would not favor the rising temperatures associated with global climate change, and it is unlikely that the blooms would be shifted to winter because of light limitations on photosynthesis. Furthermore, higher CO<sub>2</sub> levels did not significantly increase growth or photosynthesis. It could be that blooms of *U. pertusa* in Korean waters may not, in fact, change because of constraints by temperature. This does not preclude the possibility that other algal species with naturally occurring higher temperature requirements may be a source of blooms, especially since there is no prospect for regional declines in nutrient inputs and the resulting eutrophication of near-shore coastal environments. Increased temperature not only affects organisms directly but also influences the transport of nutrients from land-based sources to aquatic environments. Thus, future studies should combine climate change and other factors like light, nutrient loads, and salinity, given that many such factors will be altered

by future CO<sub>2</sub> concentrations and consequent temperature increases. For instance, photosynthetic responses related to temperature and CO<sub>2</sub> depend on light availability (Kim et al. 2013), and net photosynthetic rate decreases with increasing temperatures at subsaturating light levels, whereas the light compensation point increases as temperature rise (Davison 1991).

In recent years, research has focused on the response of whole communities to increased CO<sub>2</sub> and temperature. Rodolfo-Metalpa et al. (2011), for example, showed that the rising temperature could aggravate the benthic communities combined with ocean acidification in the Mediterranean Sea. In the case of the phytobenthos communities, the effects of rising CO<sub>2</sub> levels singularly or combination with higher temperatures induced a variety of responses from individual or assemblages of macroalgal species, and high CO<sub>2</sub> and high temperature were shown to reduce macroalgal assemblage biomass (Olabarria et al. 2013). There may also be a synergistic positive effect of high CO<sub>2</sub> and high temperature on algal turfs, but changes in the algal turfs are also exacerbated kelp loss (Connell and Russell 2010). As mentioned above, the combined effects of CO<sub>2</sub> and temperature vary and are complex at both the individual and community levels. Our research is relevant to understanding the changes in photosynthetic characteristics and growth responses of a coastal bloom-forming algal species with respect to future climate change as represented by ocean acidification and global warming.

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