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# The short-term effects of elevated CO<sub>2</sub> and ammonium concentrations on physiological responses in *Gracilariopsis lemaneiformis* (Rhodophyta)

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## Abstract

Ocean acidification (OA) and coastal eutrophication affect coastal marine organisms. We studied the physiological responses of *Gracilariopsis lemaneiformis* (Gracilariales, Rhodophyta) to increased concentrations of CO<sub>2</sub> and NH<sub>4</sub><sup>+</sup>. Incubation treatments were applied at two different pH units (low, 7.5; high (control), 7.9) and three different NH<sub>4</sub><sup>+</sup> concentrations (low, 10; medium, 50; high, 100 μM). Growth, rates of photosynthetic oxygen evolution, and NH<sub>4</sub><sup>+</sup> uptake rates were affected by both elevated CO<sub>2</sub> and NH<sub>4</sub><sup>+</sup> conditions. The changes in the pH of culture media were influenced by elevated CO<sub>2</sub> or NH<sub>4</sub><sup>+</sup> treatments. However, chlorophyll fluorescence was affected only by the level of NH<sub>4</sub><sup>+</sup>. These results indicate that the physiological responses of *G. lemaneiformis* might be enhanced when the concentrations of CO<sub>2</sub> and NH<sub>4</sub><sup>+</sup> rise. Therefore, cultures of this alga could provide a good mitigation solution against ongoing problems with OA and coastal eutrophication.

**Keywords:** CO<sub>2</sub>, Eutrophication, *Gracilariopsis lemaneiformis*, NH<sub>4</sub><sup>+</sup>, Ocean acidification (OA)

## Background

In coastal regions, marine organisms face serious environmental problems caused by anthropogenic eutrophication and, more recently, ocean acidification, or OA (Fei 2004; Doney et al. 2009; Boyd 2011). The combinations of ocean acidification and changing states of eutrophication affect the physiology of marine species (Reymond et al. 2013; Chen et al. 2016). A report by the Intergovernmental Panel on Climate Change (IPCC 2014) revealed that, since the Industrial Revolution, atmospheric CO<sub>2</sub> concentrations have increased from 280 to 400 ppm while pH values have decreased 0.1 units. Orr et al. (2005) have predicted that pH values will decline by 0.3 to 0.4 units if atmospheric CO<sub>2</sub> concentrations reach above 800 ppm. This OA phenomenon influences the physiology of marine biota such as calcifying species, phytoplankton, and

seaweeds (Hinga 2002; Gao and Zheng 2010; Cornwall et al. 2012; Kram et al. 2016). Although OA can have a negative impact on the metabolism of calcifying organisms (Hofmann and Bischof 2014; Kram et al. 2016), many macroalgae respond positively under acidified conditions (Zou 2005; Sarker et al. 2013; Kram et al. 2016). For example, many macroalgal species utilize carbon-concentrating mechanisms (CCMs) that enable them to acquire CO<sub>2</sub> from HCO<sub>3</sub><sup>-</sup>. When concentrations of dissolved inorganic carbon (DIC) are elevated, macroalgae take up this DIC and conserve energy by down-regulating their CCMs (Beardall et al. 1998; Sarker et al. 2013). In this process, the growth of seaweeds might be enhanced under higher CO<sub>2</sub> concentrations (Beardall et al. 1998; Sarker et al. 2013).

Coastal eutrophication is a severe challenge that has resulted from human activities such as aquaculture, industrialization, and urbanization. Higher concentrations of nutrients, e.g., nitrogen (N) and phosphorus (P), can improve photosynthetic rates in primary producers, including seaweeds (Chen et al. 2016; Xu et al. 2017b). However, excessive inputs of those nutrients can change

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the function and structure of a marine ecosystem (Valiela et al. 1997; Wu et al. 2015). In addition, eutrophication could also cause ocean acidification in the coastal zones because of the active respiration of bacteria (Cai et al. 2011).

*Gracilariopsis lemaneiformis* (Bory de Saint-Vincent) E.Y.Dawson, Acleto & Foldvik (Gracilariales, Rhodophyta, formerly *Gracilaria lemaneiformis*) is distributed in tropical and warm-temperate regions, and is cultivated worldwide, especially in Chile, China, and Taiwan (Armisen 1995; Tseng 2001; Yang et al. 2015). In China, this alga is cultivated on a large scale because it is an economically important source of agar (Fei 2004; Wang et al. 2010). Furthermore, this species has been studied as a valuable component of Integrated Multi-Trophic Aquaculture and as a bioremediation agent (Yang et al. 2006, 2015; Wu et al. 2015).

The physiological responses of *G. lemaneiformis* have previously been monitored in experiments that combined various CO<sub>2</sub> levels with a range of nutrient concentrations or light intensities (Zou and Gao 2009; Xu et al. 2010; Xu and Gao 2012; Chen et al. 2017). The results from those studies indicated that plant growth, photosynthesis, rate of nutrient uptake, and levels of biochemical components and amino acids are increased under elevated CO<sub>2</sub> conditions, light intensities, or nutrient concentrations (Zou and Gao 2009; Xu et al. 2010; Xu and Gao 2012; Chen et al. 2017). However, that earlier research did not focus on the physiological reactions of *G. lemaneiformis* under higher CO<sub>2</sub> and ammonium (NH<sub>4</sub><sup>+</sup>) concentrations. Therefore, we looked at whether this species has the capacity to mitigate the effects of OA and coastal eutrophication by measuring growth rates, pH changes in the culture medium, oxygen evolution by photosynthesis, the rate of ammonium uptake (an indicator of plant capacity for nutrient removal), and chlorophyll fluorescence (for assessing photosynthetic efficiency). We then analyzed the interactions among different combinations of CO<sub>2</sub> and NH<sub>4</sub><sup>+</sup> levels.

## Methods

We collected *Gracilariopsis lemaneiformis* at culture farm near Nanao Island, Shantou, China (23° 20' N,

116° 55' E), in March of 2016. Samples were transported to the field station near the farm and washed several times to remove any epiphytes. They were acclimated for 24 h before the experiments under 20 °C, 100 μmol photons m<sup>-2</sup> s<sup>-1</sup> (12:12 light/dark; a LI-250 light meter, LI-COR, USA). For each treatment, fresh samples (2 g) were placed in culture vessels containing 250 mL of filtered seawater. All experiments utilized a factorial design that included two pH conditions (low 7.5; high 7.9, control) and three ammonium concentrations (low 10; medium 50; high 100 μM). The low NH<sub>4</sub><sup>+</sup> level was based on the ambient NH<sub>4</sub><sup>+</sup> (8.84 ± 1.42 μM) concentration at the sampling site. The medium and high NH<sub>4</sub><sup>+</sup> concentrations were assumed severe eutrophication conditions and were made by adding to ambient NH<sub>4</sub><sup>+</sup> level, respectively. The low-pH condition indicated elevated CO<sub>2</sub> concentration in the future condition and high-pH condition has shown the ambient CO<sub>2</sub> level. The six culture treatments (four replicates each) were identified as follows: L<sub>pH</sub>L<sub>A</sub>, low pH + low NH<sub>4</sub><sup>+</sup>; H<sub>pH</sub>L<sub>A</sub>, high pH + low NH<sub>4</sub><sup>+</sup>; L<sub>pH</sub>M<sub>A</sub>, low pH + medium NH<sub>4</sub><sup>+</sup>; H<sub>pH</sub>M<sub>A</sub>, high pH + medium NH<sub>4</sub><sup>+</sup>; L<sub>pH</sub>H<sub>A</sub>, low pH + high NH<sub>4</sub><sup>+</sup>; and H<sub>pH</sub>H<sub>A</sub>, high pH + high NH<sub>4</sub><sup>+</sup>. The low-pH medium was prepared by mixing filtered seawater with pure CO<sub>2</sub>-enriched seawater from a CO<sub>2</sub> tank. Gattuso et al. (2010) indicated that mixing of high CO<sub>2</sub> seawater is the reasonable method as similar as CO<sub>2</sub> bubbling. The medium and high NH<sub>4</sub><sup>+</sup> concentrations were obtained by adding 50 or 100 μM NH<sub>4</sub>Cl, respectively, to the medium. All media were changed every day. Total alkalinity (TA) was measured by the electro-titration method (Gran 1952; precision ±4 μmol kg<sup>-1</sup>). The amounts of DIC and the pCO<sub>2</sub> values were calculated by the CO2SYS program (Lewis and Wallace 1998) (Table 1). The dissociation constant and KSO<sub>4</sub> values were defined by Millero et al. (2006) and Dickson (1990).

The growth of *G. lemaneiformis* was quantified by measuring the change in fresh weight (FW) after 3 days of incubation. The relative growth rate (RGR; % day<sup>-1</sup>) was calculated as follows:

**Table 1** Parameters of seawater carbonate system under tested pH conditions

Culture conditions	TA	pCO <sub>2</sub>	CO <sub>2</sub>	CO <sub>3</sub> <sup>2-</sup>	HCO <sub>3</sub> <sup>-</sup>	DIC
L <sub>pH</sub> L <sub>A</sub>	2012.75 ± 1.77	1420.09 ± 1.27	46.11 ± 0.04	52.60 ± 0.05	1878.61 ± 1.67	1977.32 ± 1.76
L <sub>pH</sub> M <sub>A</sub>	2017.33 ± 1.87	1423.37 ± 1.34	46.22 ± 0.04	52.72 ± 0.05	1882.94 ± 1.77	1981.88 ± 1.87
L <sub>pH</sub> H <sub>A</sub>	2015.42 ± 6.29	1405.93 ± 3.86	45.60 ± 0.13	53.76 ± 0.13	1879.16 ± 5.03	1978.52 ± 5.29
H <sub>pH</sub> L <sub>A</sub>	2022.16 ± 2.60	394.69 ± 0.53	12.82 ± 0.02	146.20 ± 0.20	1651.11 ± 2.21	1810.13 ± 2.42
H <sub>pH</sub> M <sub>A</sub>	2021.86 ± 2.40	394.63 ± 0.49	12.81 ± 0.02	146.18 ± 0.18	1650.86 ± 2.04	1809.85 ± 2.23
H <sub>pH</sub> H <sub>A</sub>	2019.51 ± 5.00	389.79 ± 1.35	12.69 ± 0.04	148.36 ± 0.28	1645.46 ± 4.43	1806.51 ± 4.76

Values are means ± SD. Both pH and TA (μmol kg<sup>-1</sup>) were measured directly for each scenario, whereas pCO<sub>2</sub> (μatm), CO<sub>2</sub> (μmol kg<sup>-1</sup>), CO<sub>3</sub><sup>2-</sup> (μmol kg<sup>-1</sup>), HCO<sub>3</sub><sup>-</sup> (μmol kg<sup>-1</sup>), and DIC (μmol kg<sup>-1</sup>) were calculated according to the CO2SYS program of Lewis and Wallace (1998)

$$RGR = (\ln W_2 - \ln W_1) \times 100 \times T^{-1}$$

where  $W_1$  is the initial fresh weight,  $W_2$  is the final fresh weight, and  $T$  is the incubation period (3 days).

Over a 12-h period, we monitored changes in pH in the culture media, the rate of oxygen evolution, and  $NH_4^+$  uptake. An Orion-250A meter (ThermoScientific, USA) was used to measure pH at 0, 2, 4, 6, and 12 h. We also assessed the changes in pH in vessels containing media but no specimens (blanks), under low- and high-pH conditions.

Rates of photosynthetic oxygen evolution ( $\mu\text{mol O}_2 \text{ g}^{-1} \text{ FW h}^{-1}$ ) were recorded with a Clark-type microelectrode oxygen sensor (Unisense, Denmark), which was calibrated with a mixture of  $C_6H_7NaO_6$  (sodium ascorbate) of 0.4 g and NaOH (sodium hydroxide) of 2 g in the 100-mL dilution water.

The rate of  $NH_4^+$  uptake ( $\mu\text{mol NH}_4^+ \text{ g}^{-1} \text{ FW h}^{-1}$ ) was calculated as the amount lost from each culture medium over 12 h. The measurement method of  $NH_4^+$  uptake rates were described by Parsons et al. (1984). The following equation was used in the calculation:

$$V = (S_i - S_f) \times \text{vol} \times W^{-1} \times T^{-1}$$

where  $S_i$  is the initial concentration of  $NH_4^+$ ,  $S_f$  is the final concentration after  $T$  hours of incubation, vol is the volume of the medium, and  $W$  is the fresh weight of each algal specimen.

Chlorophyll fluorescence was determined after 3 days with a Plant Efficiency Analyzer (PEA, Hansatech, UK). The maximum quantum yield ( $F_v/F_m$ ) of Photosystem II was calculated as follows:

$$F_v/F_m = (F_m - F_o)/F_m$$

where  $F_m$  is maximum fluorescence after dark adaptation and  $F_o$  is minimum fluorescence after dark adaptation. Algal samples were placed in leaf-clip holders and  $F_v/F_m$  was measured by applying a saturating pulse after the samples were dark-adapted for 15 min.

One- and two-way ANOVA were conducted with all experimental data. Normality and homogeneity were investigated before the statistical analysis began. After that, Tukey's tests were used to compare among treatments, and the threshold for statistically significant differences was set at  $p < 0.05$ . All analyses were performed with the SPSS software program (version 23.0).

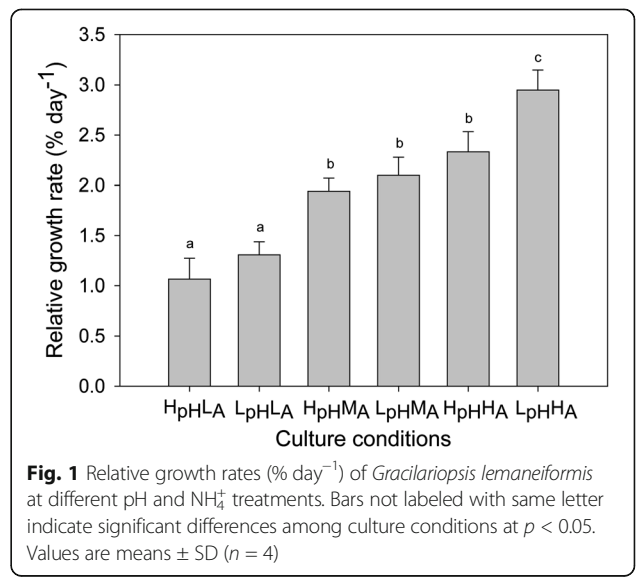
**Results**

Results of physiological responses of *Gracilariopsis lemaneiformis* under elevated  $CO_2$  and  $NH_4^+$  treatments were summarized in Table 2. During the incubation period, the relative growth rate of *Gracilariopsis lemaneiformis* increased under elevated  $CO_2$  and  $NH_4^+$  treatments. The

**Table 2** Results from two-way ANOVA of physiological activities (relative growth rate (% day<sup>-1</sup>), extent of variation in pH, photosynthetic oxygen evolution rate ( $\mu\text{mol O}_2 \text{ g}^{-1} \text{ FW h}^{-1}$ ), ammonium uptake rate ( $\mu\text{mol NH}_4^+ \text{ g}^{-1} \text{ FW h}^{-1}$ ), and photosynthetic efficiency ( $F_v/F_m$ )) by samples of *Gracilariopsis lemaneiformis*

Parameter/source of variation	df	MS	F value	p value
Relative growth rate				
pH level	1	0.69	21.50	<0.01
$NH_4^+$ concentration	2	4.26	132.54	<0.01
pH level $\times$ $NH_4^+$ concentration	2	0.12	3.66	0.04
Extent of variation in pH				
pH level	1	1.09	166.48	<0.01
$NH_4^+$ concentration	2	0.38	57.63	<0.01
pH $\times$ $NH_4^+$	2	0.01	0.10	0.90
Photosynthetic oxygen evolution rate				
pH level	1	198.72	68.85	<0.01
$NH_4^+$ concentration	2	3678.60	1274.53	<0.01
pH $\times$ $NH_4^+$	2	22.50	7.80	<0.01
$NH_4^+$ uptake rate				
pH level	1	0.17	24.72	<0.01
$NH_4^+$ concentration	2	85.78	12,108.59	<0.01
pH $\times$ $NH_4^+$	2	0.13	18.95	<0.01
Photosynthetic efficiency				
pH level	1	<0.01	0.58	0.46
$NH_4^+$ concentration	2	0.13	51.60	<0.01
pH $\times$ $NH_4^+$	2	<0.01	0.92	0.42

maximum RGR was  $2.95 \pm 0.20\% \text{ day}^{-1}$  under  $L_{pH}H_A$  conditions while the minimum RGR,  $1.07 \pm 0.21\% \text{ day}^{-1}$ , was achieved under  $H_{pH}L_A$  conditions (Fig. 1). At the high  $NH_4^+$  levels, RGR was greater at pH 7.5 than at pH 7.9 ( $H_A$ :  $F = 6.04, p = 0.04$ ).



Alterations in pH in the culture media containing algal specimens were significantly affected by initial pH or  $\text{NH}_4^+$  levels. The extent of variation in pH ranged from  $0.44 \pm 0.02$  to  $1.26 \pm 0.18$  units, and the maximum and minimum changes in pH were associated with  $L_{\text{pH}}H_A$  and  $H_{\text{pH}}L_A$ , respectively (Fig. 2; Table 3). At each pH level, the medium and high  $\text{NH}_4^+$  concentrations were significantly different from the low  $\text{NH}_4^+$  condition ( $L_{\text{pH}}$ :  $F = 17.08$ ,  $p = 0.01$ ;  $H_{\text{pH}}$ :  $F = 78.98$ ,  $p < 0.01$ ). At individual  $\text{NH}_4^+$  levels, the change in pH was significantly greater in the low-pH treatment than in the high-pH treatment ( $L_A$ :  $F = 6.65$ ,  $p = 0.04$ ;  $M_A$ :  $F = 6.94$ ,  $p = 0.04$ ;  $H_A$ :  $F = 138.86$ ,  $p < 0.01$ ). The pH values in blank culture media were remain constant over 12 h that pH values were affected only photosynthesis oxygen evolution of *G. lemaneiformis*.

Photosynthetic oxygen evolution was affected by elevated levels of  $\text{CO}_2$  and  $\text{NH}_4^+$ . Rates were  $62.28 \pm 1.71 \mu\text{mol O}_2 \text{ g}^{-1} \text{ FW h}^{-1}$  under  $H_{\text{pH}}L_A$  and  $111.48 \pm 0.95 \mu\text{mol O}_2 \text{ g}^{-1} \text{ FW h}^{-1}$  under  $L_{\text{pH}}H_A$  (Fig. 3). When compared at the same initial pH, those rates increased significantly as the  $\text{NH}_4^+$  level rose ( $L_{\text{pH}}$ :  $F = 479.22$ ,  $p < 0.01$ ;  $H_{\text{pH}}$ :  $F = 854.92$ ,  $p < 0.01$ ). Under medium and high  $\text{NH}_4^+$  conditions, the rate was greater at

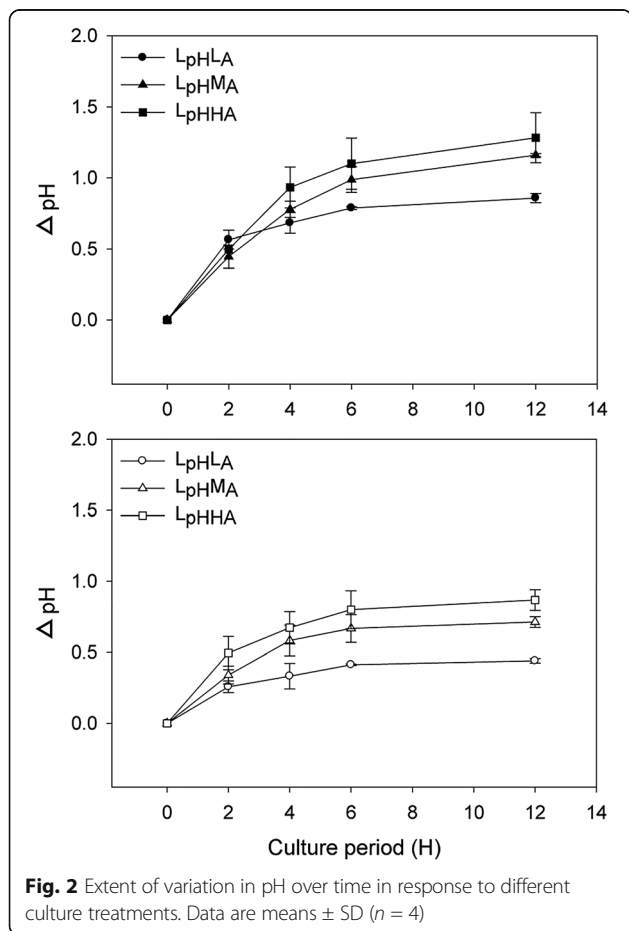
low than at high pH but there was no significant difference at the low  $\text{NH}_4^+$  level ( $L_A$ :  $F = 0.26$ ,  $p = 0.63$ ;  $M_A$ :  $F = 7.94$ ,  $p = 0.03$ ;  $H_A$ :  $F = 7.67$ ,  $p = 0.03$ ).

The rate of  $\text{NH}_4^+$  uptake was significantly affected by elevated concentrations of  $\text{CO}_2$  and  $\text{NH}_4^+$ , with rates ranging from  $0.84 \pm 0.01$  to  $7.43 \pm 0.03 \mu\text{mol NH}_4^+ \text{ g}^{-1} \text{ FW h}^{-1}$  (Fig. 4). Uptake was more rapid under  $L_{\text{pH}}H_A$  conditions and slowest under  $H_{\text{pH}}L_A$  treatment. When those rates were compared at the same pH, values increased under higher  $\text{NH}_4^+$  concentrations ( $L_{\text{pH}}$ :  $F = 3230.83$ ,  $p < 0.01$ ;  $H_{\text{pH}}$ :  $F = 25,898.16$ ,  $p < 0.01$ ). Furthermore, at the same level of  $\text{NH}_4^+$ , uptake was more rapid under  $L_{\text{pH}}H_A$  than under  $H_{\text{pH}}H_A$  treatment ( $H_A$ :  $F = 6.50$ ,  $p = 0.04$ ).

When values of the chlorophyll fluorescence were used as a proxy to represent photosynthetic efficiency, they ranged from  $0.55 \pm 0.22$  ( $H_{\text{pH}}L_A$ ) to  $0.64 \pm 0.02$  ( $L_{\text{pH}}H_A$ ) (Fig. 5). Although values of  $F_v/F_m$  were significantly affected under high  $\text{NH}_4^+$  levels and under both pH conditions ( $L_{\text{pH}}$ :  $F = 44.64$ ,  $p < 0.01$ ;  $H_{\text{pH}}$ :  $F = 15.91$ ,  $p < 0.01$ ), they were not significantly influenced by  $\text{NH}_4^+$  conditions ( $L_A$ :  $F = 0.60$ ,  $p = 0.47$ ;  $M_A$ :  $F = 1.23$ ,  $p = 0.31$ ;  $H_A$ :  $F = 0.92$ ,  $p = 0.37$ ).

## Discussion

Growth of *Gracilariopsis lemaneiformis* was affected by elevated  $\text{CO}_2$  and  $\text{NH}_4^+$  treatments and there were interactions of both factors. Previous studies have revealed similar results with *G. lemaneiformis*, *Hypnea spinella*, *Chondrus crispus*, *Pyropia haitanensis*, and *Ulva pertusa* (Yu and Yang 2008; Suárez-Álvarez et al. 2012; Sarker et al. 2013; Chen et al. 2016; Kang and Chung 2017). When DIC concentrations rise in the ocean, many macroalgal species conserve energy by regulating their CCMs, thereby improving their growth performance (Sarker et al. 2013). For example, red algae within the *Gracilaria* genus used external carbonic anhydrase to increase their capacity for  $\text{HCO}_3^-$  utilization, which boosts their growth under elevated  $\text{CO}_2$  conditions (Israel and Beer 1992; García-Sánchez et al. 1994; Zou et al. 2004). Greater availability of nutrients can also enhance the growth of macroalgae (Yu and Yang 2008). Although greater accumulations of nitrogen can stimulate growth, Xu et al. (2010) reported that *G. lemaneiformis* development was influenced by either  $\text{CO}_2$  or the level of phosphorus, but those two factors did not show synergistic effects. Our results indicated that RGR were lower than those of other experiments. We speculated that short-term incubation period and high stocking density could be the reason of lower RGR. Kim et al. (2013) indicated that high stocking density might cause lower growth rates than those of light shading of the culture vessels. We assumed the high stocking density conditions of aquaculture farm to find realistic growth of *G.*





**Table 3** Maximum pH values and extent of variation in response over 12-h period to different treatment combinations

	Maximum pH values		Extent of variation	
	L <sub>pH</sub> (low pH)	H <sub>pH</sub> (high pH)	L <sub>pH</sub> (low pH)	H <sub>pH</sub> (high pH)
L <sub>A</sub> (low NH <sub>4</sub> <sup>+</sup> )	8.36 ± 0.03 <sup>a</sup>	8.34 ± 0.02 <sup>a</sup>	0.86 ± 0.03 <sup>a</sup>	0.44 ± 0.02 <sup>b</sup>
M <sub>A</sub> (medium NH <sub>4</sub> <sup>+</sup> )	8.66 ± 0.01 <sup>b</sup>	8.61 ± 0.04 <sup>b</sup>	1.16 ± 0.01 <sup>c</sup>	0.71 ± 0.04 <sup>a</sup>
H <sub>A</sub> (high NH <sub>4</sub> <sup>+</sup> )	8.78 ± 0.18 <sup>b</sup>	8.77 ± 0.07 <sup>b</sup>	1.28 ± 0.18 <sup>c</sup>	0.87 ± 0.07 <sup>a</sup>

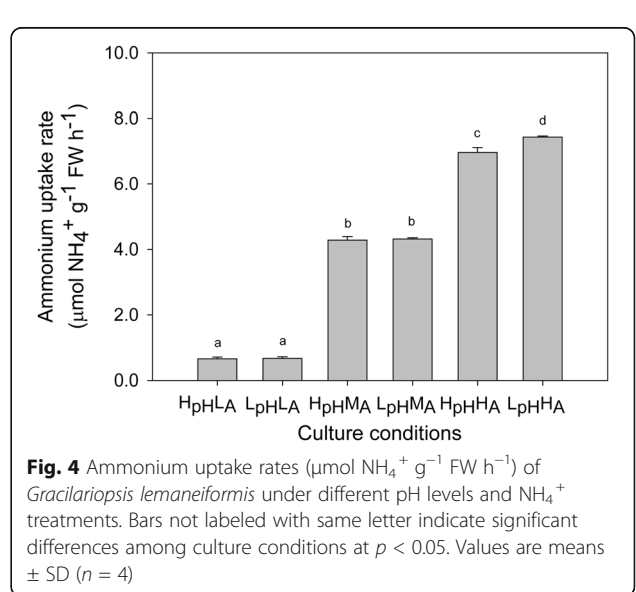
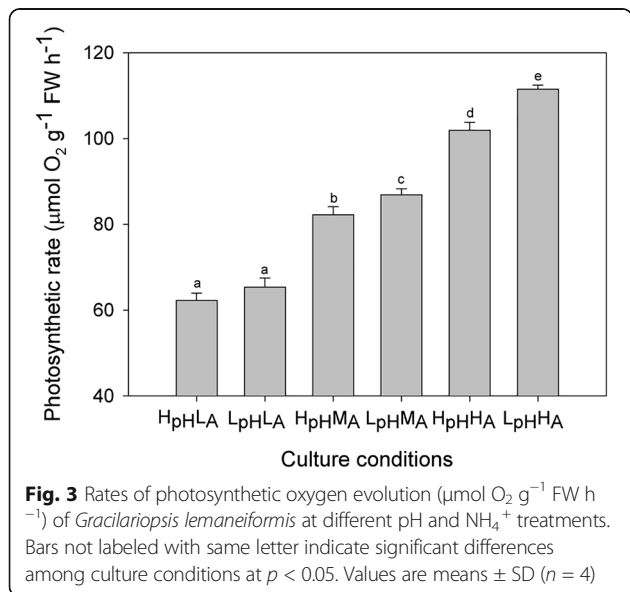
Data are means ± SD (n = 4). Within a column, values not followed by the same letter are significantly different at p < 0.05

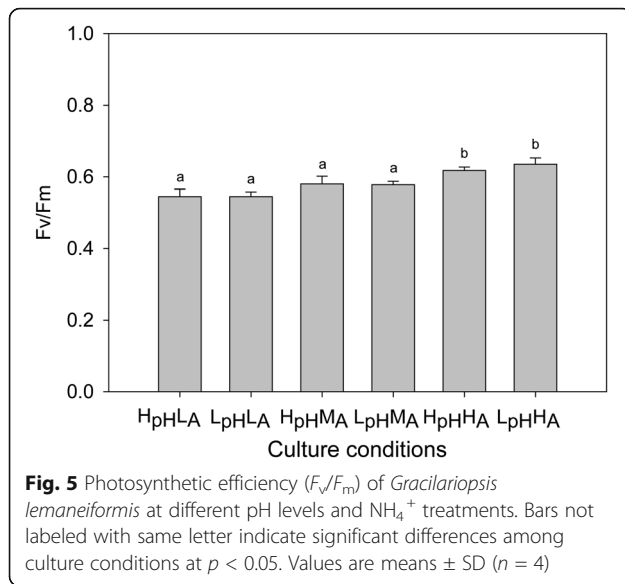
*lemaniformis*. In addition, Xu et al. (2017a) found that the RGRs of short-time cultivation period were higher than those of long-time cultivation time. They explained that decreased of RGRs resulted from the acclimation to culture conditions.

The pH values in the culture medium could be used as an indicator of physiological characteristics of the macroalgae (Maberly 1990; Murru and Sandgren 2004). The pH of our culture medium was altered in response to increases in CO<sub>2</sub> or NH<sub>4</sub><sup>+</sup> concentrations. The addition of CO<sub>2</sub> gas caused pH values to decline while the concentration of DIC, such as CO<sub>2</sub>(aq), and HCO<sub>3</sub><sup>-</sup> increased. This change in pH was more dramatic in the low-pH medium than under high-pH conditions. The DIC concentrations were elevated in the culture medium, which lead to increases in pH levels by photosynthesis (Zhang et al. 2012). In addition, we did not find any inhibition of photosynthesis. When pH values are >9.0, photosynthesis of some species is more likely to be inhibited (Maberly 1990; Björk et al. 2004). Zou et al. (2004) indicated that inorganic carbon (Ci) affinity and photosynthetic rates at pH 9.0 were decreased in *G. lemaneiformis*. In case of pH values over 9.0, this alga does not accumulate CO<sub>2</sub> because of poor capacity of HCO<sub>3</sub><sup>-</sup> utilization (Zou et al. 2004).

Photosynthetic oxygen evolution in our *Gracilariopsis lemaneiformis* samples was also increased by elevated CO<sub>2</sub> and NH<sub>4</sub><sup>+</sup> levels. The same has been reported for *Hizikia fusiforme* (currently, *Sargassum fusiforme*), *Hypnea spinella*, and *Pyropia haitanensis* (Zou 2005; Suárez-Álvarez et al. 2012; Chen et al. 2016). Zou et al. (2004) showed that the photosynthesis of *G. lemaneiformis* is already saturated under normal DIC concentrations found in natural seawater. However, we noted that this species was also influenced by the synergism between CO<sub>2</sub> and NH<sub>4</sub><sup>+</sup>. Similarly, Xu et al. (2010) has shown that the maximum net photosynthetic rate for *G. lemaneiformis* is influenced by increases in both CO<sub>2</sub> and phosphorus concentrations.

The uptake of NH<sub>4</sub><sup>+</sup> was influenced by treatment with exogenous CO<sub>2</sub> and NH<sub>4</sub><sup>+</sup>. Research with *Gracilaria* sp., *G. chilensis*, *Hizikia fusiforme*, *Gracilariopsis lemaneiformis*, *Pyropia yezoensis*, *P. haitanensis*, and *U. pertusa* has demonstrated that all respond to elevated levels of CO<sub>2</sub> or NH<sub>4</sub><sup>+</sup> (Gao et al. 1993; Zou 2005; Xu et al. 2010; Kang et al. 2014; Chen et al. 2016; Kang and Chung 2017). Increasing the concentration of CO<sub>2</sub> can enhance nitrogen uptake and the activity of nitrate reductase (Gordillo et al. 2001; Xu et al. 2010; Hofmann et al. 2013; Liu and Zou 2015). In various seaweeds, the uptake of NH<sub>4</sub><sup>+</sup> is greater when the initial NH<sub>4</sub><sup>+</sup>





concentration is high (Dy and Yap 2001). In addition, when more nutrients are available, they are more easily absorbed (Runcie et al. 2003; Pérez-Mayorga et al. 2011). Therefore, because  $\text{NH}_4^+$  uptake by our *G. lemaneiformis* samples was more rapid under the elevated  $\text{CO}_2$  and  $\text{NH}_4^+$  treatment, we speculate that this was due to the associated rise in photosynthesis.

Chlorophyll fluorescence, which can reflect photosynthetic efficiency, is enhanced by increased N concentrations (Dawes and Koch 1990). However, the role of  $\text{CO}_2$  is debatable with some studies showing that  $F_v/F_m$  is not affected by elevated  $\text{CO}_2$  levels (Hofmann et al. 2012; Olischläger et al. 2013; Kram et al. 2016) and others indicating that chlorophyll fluorescence increases under high  $\text{CO}_2$  concentrations (Chen et al. 2015). We found here that the photosynthetic efficiency of *G. lemaneiformis* was more strongly affected by greater  $\text{NH}_4^+$  concentrations than by higher  $\text{CO}_2$  levels. Because of these contrasting reports, direct measurement of oxygen evolution is now considered a more appropriate method than chlorophyll fluorescence for monitoring changes in efficiency (Kram et al. 2016).

Our data provided evidence that the physiological activities of *G. lemaneiformis* are enhanced under elevated  $\text{CO}_2$  and  $\text{NH}_4^+$  treatments. We predict that mass production of this species in China will increase in response to OA and eutrophication. Kim and Yarish (2014) indicated that productivity of *Gracilaria* sp. was enhanced under elevated  $\text{CO}_2$  levels with high stocking density and irradiance. The biomass production of *G. chilensis* was also increased at elevated  $\text{CO}_2$  levels or high nutrient concentrations (Buschmann et al. 1994). The increase of harvest can be used as agar materials, seafood, and feed for abalone (Tseng 2001; Fei 2004; Yang et al.

2015). Yang et al. (2015) have suggested that large-scale cultivation of *Gracilaria* can improve water quality by uptake of excessive nutrients and potential carbon sink absorber along the coast of China. Therefore, this alga could provide a good solution for mitigation against the problems associated with such marine challenges. If we are to achieve practical results, future investigations will require more large-scale, long-term experiments. Those projects must also focus on the synergistic effects of several environmental factors, e.g., temperature, light intensity, level of salinity, and nutrient concentrations.

## Conclusions

The combined treatment of elevated  $\text{CO}_2$  and  $\text{NH}_4^+$  heightened the physiological reactions of *G. lemaneiformis*, as demonstrated by changes in relative growth rates, rates of photosynthetic oxygen evolution, and the uptake of ammonium. The pH values were affected each elevated  $\text{CO}_2$  or  $\text{NH}_4^+$  treatments. In contrast, we noted that chlorophyll fluorescence was affected only by altering the concentration of  $\text{NH}_4^+$ .

## Abbreviations

OA: Ocean acidification; RGR: Relative growth rates

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## Availability of data and materials

All datasets in this study are available from the corresponding author on reasonable request.

## Authors' contributions

All authors conducted the field survey in the sampling stations. JW, Cicilia, and IK conducted experiments and wrote manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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