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The effects of eutrophication and acidification on the ecophysiology of *Ulva pertusa* Kjellman

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Abstract In coastal environments, acidification and eutrophication affect the physiology of marine macroalgae. We investigated the responses of *Ulva pertusa* Kjellman (Ulvales, Chlorophyta) under such conditions. Samples were cultured at two different pH settings (low, 7.5; high, 8.0) and at three different ammonium levels (low, 4; medium, 60; high, 120 μM NH_4^+). Our objective was to analyze the influence that elevated CO_2 and NH_4^+ might have on pH, oxygen evolution, rates of nutrient uptake, chlorophyll fluorescence, growth, and C/N ratio of that organism. Variability in pH value was enhanced under low pH/high NH_4^+ and was significantly different ($p < 0.05$) from changes measured when the high pH/low NH_4^+ combination was applied. Rates of NH_4^+ uptake and relative growth rates by *U. pertusa* were increased under low pH/high NH_4^+ conditions and that response was significantly different ($p < 0.05$) from the other treatments. The rate of photosynthetic oxygen evolution and chlorophyll fluorescence were increased under elevated NH_4^+ concentrations ($p < 0.05$). However, the C/N ratio of *U. pertusa* was not affected by higher concentrations of CO_2 and NH_4^+ ($p > 0.05$). Our results indicated that the physiological reactions of this alga were heightened when exposed to either the elevated combination of CO_2/NH_4^+ or even when only the level of NH_4^+ was raised. Although such excessive growth can lead to bloom formations in coastal areas, this species also has greater capacity for taking up nutrients and dissolved inorganic carbon.

Keywords Ammonium (NH_4^+) · Carbon dioxide (CO_2) · Eutrophication · Ocean acidification (OA) · *Ulva pertusa* · Chlorophyceae

Introduction

Because continuous expansion of industrial activities since the Industrial Revolution has affected coastal environments, many coastal organisms are being challenged by anthropogenic changes such as acidification and eutrophication. The combination of these two environmental phenomena can have individual influences as well as synergistic interactions with marine species (Horta et al. 2012; Reymond et al. 2013). The effects of eutrophication have been studied in various marine organisms, but our current examination focused first on the influence of ocean acidification (OA). The Intergovernmental Panel on Climate Change (IPCC 2014) has projected that atmospheric CO_2 concentrations will increase by 280 to 400 ppm. Caldeira and Wickett (2005) also have predicted decreases in pH values of 0.3 to 0.4 units by the year 2100 and 0.7 to 0.8 units by the year 2300. Ocean acidification presents a severe challenge to the physiology and ecology of marine ecosystems in coastal areas and can have a negative effect on the metabolism of marine organisms (Kroeker et al. 2013; Cornwall and Hurd 2015; Kram et al. 2016). However, some macroalgal species can also show a positive response to acidification (Hurd et al. 2009; Suárez-Álvarez et al. 2012; Sarker et al. 2013) because of their unique carbon-concentrating mechanisms (CCMs) (Raven et al. 2011). Such macroalgae use CCMs that acquire carbon either directly from bicarbonate or via the carbonic anhydrase enzyme

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(Raven 1997; Giordano et al. 2005). Those processes could enhance algal growth because of the energy saved from dissolved inorganic carbon (DIC) transport (Beardall et al. 1998; Beardall and Giordano 2002; Wu et al. 2008).

Cultural eutrophication is another serious environmental issue that is promoted by anthropogenic activities such as urbanization, inflow of waste water, and aquaculture. Excessive input of nutrients can lead to massive algal blooms and induce bacterial respiration. This active respiration can lead to a state of OA because of elevated CO_2 levels in the water (Cai et al. 2011; Sunda and Cai 2012). Eutrophication also damages marine communities and reduces species diversity (Lohman and Priscu 1992; Valiela et al. 1997; de Faveri et al. 2015). Nevertheless, some photosynthetic macroalgae, e.g., *Ulva* spp., have a metabolic advantage under enriched nutrient conditions (Ye et al. 2011; Luo et al. 2012).

In terms of functional form and high rates of growth and nutrient uptake, *Ulva* spp. are opportunistic because of their thin sheet-like thallus (Littler 1980; Cohen and Fong 2006). Worldwide, green tides that result from excessive algal growth and blooms have a negative ecological impact because the decomposition of biomass into the water column decreases oxygen levels and affects the productivity and biodiverse of the local community (Valiela et al. 1997; Hiraoka et al. 2004; Morand and Merceron 2005; Wang et al. 2009). However, these *Ulva* spp. might be beneficial because they can function in bioremediation and as a component of Integrated Multi-Trophic Aquaculture systems (Bolton et al. 2009; Ben-Ari et al. 2014).

Numerous studies have investigated the physiological responses of *Ulva* sp. under different culture conditions that manipulate CO_2 levels, nutrient concentrations, and temperature (Figueroa et al. 2014a, b; Stengel et al. 2014). However, little research has been done using *U. pertusa* and a combination of acidification and eutrophication conditions. Here, we examined the physiological responses of that species to elevated levels of CO_2 and ammonium (NH_4^+), as well as any possible interactions among different combinations of CO_2 and NH_4^+ concentrations. We monitored changes in pH levels, oxygen evolution for photosynthesis, rates of NH_4^+ uptake to show the nutrient removal capacity, fresh weight for growth, chlorophyll fluorescence to show the physiological status during the experiments, and biochemical composition to reveal any change of C/N ratio.

Materials and methods

Experiment preparation

Samples of *Ulva pertusa* were collected at Namhae, Korea (34°56′N, 127°51′E) in April of 2016. At this sampling site, the ambient temperature was approximately 14.7 ± 0.1 °C and salinity was 28.2 ± 0.2‰, as measured with a YSI Pro 2030 (USA) meter. After transport to the laboratory, they were cleaned several times with filtered seawater (0.2 μm) to remove any dirty epiphytes. The samples were then maintained in a culture room in filtered seawater under conditions of 20 °C, 80 μmol photons m^{-2} s^{-1} (12:12 light/dark), and S = 28‰. They were acclimated for 3 days before the experiments began. For each treatment, samples (3 g each) were placed in 600-mL culture beakers, each containing 500 mL of filtered seawater. In all, four replicates were made for each of the treatments. The multi-factorial design comprised two pH levels (low, 7.5; high, 8.0) and three ammonium concentrations (low, 4 μM; medium, 60 μM; high, 120 μM). The lowest pH was selected based on an ocean prediction model (Caldeira and Wickett 2005) while the ambient pH reflected conditions at the sampling site. The minimum NH_4^+ level was based on the ambient concentration (3.62 ± 0.31 μM) at the sampling site, while the medium and high NH_4^+ levels had been determined in our preliminary studies (data not shown). These treatment combinations were designated as follows: $L_{pH}L_A$, low pH/low NH_4^+; $H_{pH}L_A$, high pH/low NH_4^+; $L_{pH}M_A$, low pH/medium NH_4^+; $H_{pH}M_A$, high pH/medium NH_4^+; $L_{pH}H_A$, low pH/high NH_4^+; and $H_{pH}H_A$, high pH/high NH_4^+. The cultures were carried out at constant temperature (20 °C) along a constant-temperature table, under LED fixtures that supplied a light intensity checked with a LI-250 light meter (LI-COR, USA). We set the intensity at 80 μmol photons m^{-2} s^{-1} to prevent photochemical stress due to acidification conditions (Liu et al. 2012). Filtered seawater in the low pH culture media was injected with pure CO_2 gas from a tank. To support the low-, medium-, or high-NH_4^+ treatments, we added ammonium to the seawater to a final concentration of 4, 60, or 120 μM NH_4Cl. The medium in each treatment was replaced every 2 days and nutrients were added daily to avoid any deficiencies. Total alkalinity (TA) of the seawater was determined according to the electrotitration method (Gran 1952), at a precision of ±4 μmol kg^{-1}. The inorganic carbon concentration and pCO_2 were calculated based on pH, TA, salinity, and temperature, using the CO2SYS software program (Lewis and Wallace 1998). Dissociation constants and KSO_4 values were defined as described by Millero et al. (2006) and Dickson (1990) (Table 1).

Monitoring changes in pH, photosynthetic oxygen evolution, and rates of ammonium uptake

During the first 8 h of treatment, we measured changes in pH of the seawater medium and calculated the rates of oxygen evolution and NH_4^+ uptake. An Orion-250A pH meter (Thermoscientific, USA) was used to record pH levels at 2-h intervals for each treatment. Those values can serve as an indicator of physiological characteristics of algae under culture conditions (Maberly 1990; Murru and Sandgren 2004).

Table 1 Parameters of seawater carbonate system under different culture conditions

Culture conditions	TA	pCO_2	CO_2	CO_3^{2-}	HCO_3^-	DIC
$L_{pH}L_A$	2012.50 ± 9.57	1404.43 ± 10.61	45.61 ± 0.22	53.48 ± 0.26	1877.81 ± 9.06	1976.90 ± 9.54
$L_{pH}M_A$	2015.30 ± 7.63	1405.34 ± 11.16	45.64 ± 0.04	53.52 ± 0.40	1879.03 ± 7.54	1978.18 ± 7.63
$L_{pH}H_A$	2015.50 ± 5.57	1405.70 ± 11.35	45.65 ± 0.04	53.53 ± 0.50	1879.50 ± 7.81	1978.68 ± 7.91
$H_{pH}L_A$	2019.25 ± 8.30	389.18 ± 10.49	12.64 ± 0.05	148.20 ± 0.63	1645.52 ± 7.03	1806.36 ± 7.72
$H_{pH}M_A$	2022.05 ± 7.58	389.47 ± 10.52	12.65 ± 0.02	148.31 ± 0.20	1646.72 ± 7.19	1807.68 ± 7.40
$H_{pH}H_A$	2022.50 ± 8.29	389.57 ± 10.26	12.65 ± 0.01	148.35 ± 0.10	1647.15 ± 7.09	1808.15 ± 7.20

Values are means ± SD. Both pH and TA (μmol kg^{-1}) were measured directly for each scenario, while pCO_2 (μatm), CO_2 (μmol kg^{-1}), CO_3^{2-} (μmol kg^{-1}), HCO_3^- (μmol kg^{-1}), and DIC (μmol kg^{-1}) were calculated using the CO2SYS program (Lewis and Wallace 1998)

Dark respiration by $L_{pH}H_A$ and $H_{pH}L_A$ samples were measured because we speculated that OA is mediated by photosynthesis. Changes in pH were also investigated in beakers containing culture media without specimens (blanks), under both low- and high-pH conditions.

Photosynthetic oxygen evolution rates (μmol O_2 g^{-1} FW h^{-1}) were also analyzed with a Clark-type microelectrode oxygen sensor (Unisense, Denmark). The microelectrode was calibrated with a solution of $C_6H_7NaO_6$ (sodium ascorbate) and NaOH (sodium hydroxide) that detected response times of less than 1 s.

The rate of NH_4^+ uptake (V; μmol NH_4^+ g^{-1} FW h^{-1}) was determined based on the average amount that was removed from the culture medium during the incubation period. The measurement method of NH_4^+ uptake rates were described by Parsons et al. (1984). The following equation was used in this 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 culture, vol is the volume of the culture medium, and W is the fresh weight of each algal specimen.

Growth rates, chlorophyll fluorescence, and C/N ratio

Algal growth rates, chlorophyll fluorescence, and C/N ratio were measured after 14 days of incubation. Changes in biomass were determined at the end of the experimental period, and the relative growth rate (RGR; % day^{-1}) was calculated as follows (Yong et al. 2013):

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

where W_1 is the initial fresh weight and W_2 is the final fresh weight after T days of incubation.

Chlorophyll fluorescence was checked with a pulse amplitude modulation fluorometer (DIVING-PAM, Walz, Germany). The maximum quantum yield of Photosystem II was calculated as (Cosgrove and Borowitzka 2011):

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

where F_v is variable fluorescence, F_m is maximum fluorescence after dark-adaptation, F_o is minimum fluorescence after dark-adaptation, and F_v/F_m is photosynthetic efficiency, as measured using a saturating pulse under dark-adaptation. Samples were kept in the dark for 15 min before chlorophyll fluorescence measurement.

Tissue carbon (C) and nitrogen (N) in the alga were analyzed after the experiment. Samples were dried at 60 °C for 48 h and then ground to powder with a Tissuelyser LT (Qiagen, Germany). Approximately 2 to 3 mg of ground tissues was used with an elemental analyzer (Flash 2000 Series; Thermo Fisher Scientific, USA). The C/N ratio was calculated on a molar basis.

Statistical analysis

Two-way analysis of variance (ANOVA) was conducted with all experimental data. The distributions of values obtained for pH, rate of photosynthetic oxygen evolution, NH_4^+ uptake rate, relative growth rate, chlorophyll fluorescence, and C/N ratio were tested for normality and homogeneity before the statistical analysis began. Tukey's tests were used to compare among treatments. Differences were considered statistically significant at $p < 0.05$. All analyses were performed with the SPSS Program (version 23.0).

Results

Change in pH, photosynthetic oxygen evolution, and ammonium uptake rates

Initial pH values in the culture beakers were recorded before the experiments began. After 8 h of treatment, the maximum increase in pH was 2.76 ± 0.07 units, which occurred under

the $L_{pH}H_A$ treatment (Fig. 1), while the smallest rise, 2.12 ± 0.17 units, resulted from the $H_{pH}L_A$ combination. For pH, responses to treatment, from highest to lowest, followed the order of $L_{pH}H_A > L_{pH}M_A > L_{pH}L_A > H_{pH}H_A > H_{pH}M_A > H_{pH}L_A$. When the same NH_4^+ concentration was examined across treatments, ΔpH in low-pH treatment beakers was significantly higher than that in the high-pH beakers (Table 2). At each pH level, the change in pH under high NH_4^+ was greater, but not significantly, than levels calculated under low or medium NH_4^+ conditions (Table 2). After 8 h of treatment, pH values were not significantly different among culture conditions but all were above 10.00 units (Fig. 1, Table 3). The pH did not change over time in cultures that had no specimen (i.e., blank treatment) and values ranged from 7.50 to 7.51 under low pH conditions and from 8.00 to 8.01 for high pH conditions.

Photosynthetic oxygen evolution rates during the 8-h experiments ranged from 42.39 ± 2.49 to 69.75 ± 4.25 μmol O_2 g^{-1} FW h^{-1} (Fig. 2). When pH remained constant, photosynthetic oxygen evolution rates increased as the NH_4^+ concentration rose, and differences among treatments were significant. However, at individual NH_4^+ levels, evolution rates were not significantly different (Table 2).

Rates of NH_4^+ uptake by *Ulva pertusa* ranged from 0.08 ± 0.01 to 3.33 ± 0.10 μmol NH_4^+ g^{-1} FW h^{-1} (Fig. 3).

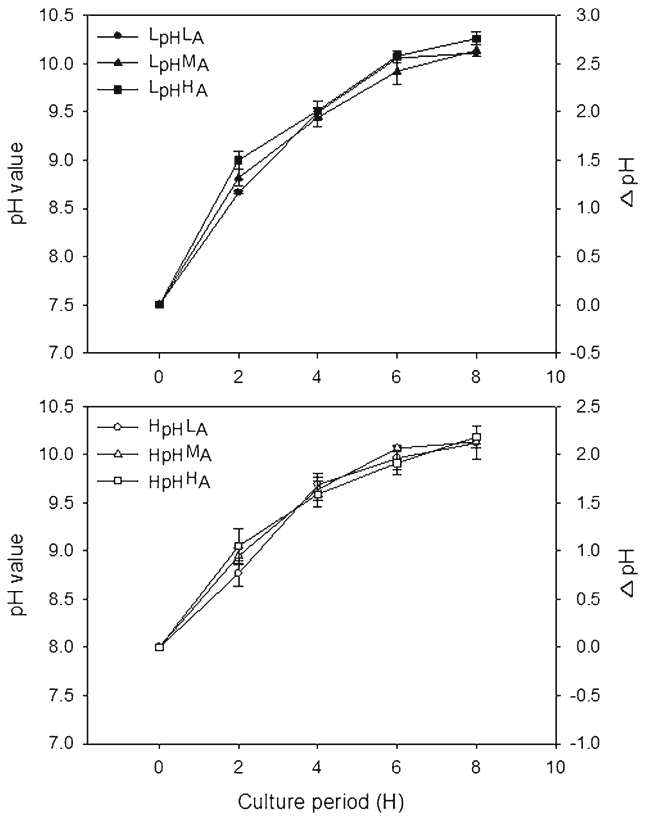

Fig. 1 Maximum values and extent of variation in pH in response to different treatment combinations over 8 h. Data are means ± SD ($n = 4$)

Table 2 Results of two-way ANOVA derived from physiological activities (extent of variation in pH, photosynthetic oxygen evolution rate, ammonium uptake rate, relative growth rate, photosynthetic efficiency, and C/N ratio) of *Ulva pertusa*

Source	*df*	MS	*F* value	*p* value
Extent of variation in pH				
pH level	1	1.62	226.29	<0.05
Ammonium concentration	2	<0.05	3.50	0.05
pH level × ammonium concentration	2	<0.05	0.59	0.57
Photosynthetic oxygen evolution rate (μmol O_2 g^{-1} FW h^{-1})				
pH level	1	134.24	4.10	0.05
Ammonium concentration	2	891.71	27.23	<0.05
pH level × ammonium concentration	2	40.66	1.24	0.31
Ammonium uptake rate (μmol NH_4^+ g^{-1} FW h^{-1})				
pH level	1	0.13	58.61	<0.05
Ammonium concentration	2	18.81	8389.01	<0.05
pH level × ammonium concentration	2	0.12	51.49	<0.05
Relative growth rate (% day^{-1})				
pH level	1	1.04	36.15	<0.05
Ammonium concentration	2	4.37	151.74	<0.05
pH level × ammonium concentration	2	0.29	10.02	<0.05
Photosynthetic efficiency (F_v/F_m)				
pH level	1	<0.05	0.04	0.84
Ammonium concentration	2	<0.05	7.48	<0.05
pH level × ammonium concentration	2	<0.05	1.31	0.30
C/N ratio (%)				
pH level	1	<0.05	0.21	0.65
Ammonium concentration	2	0.37	2.27	0.21
pH level × ammonium concentration	2	<0.05	0.15	0.87

MS means mean square

Values were highest for the $L_{pH}H_A$ samples and differences were significant among treatment combinations ($p < 0.05$). The lowest rate was 0.08 ± 0.01 μmol NH_4^+ g^{-1} FW h^{-1} for $H_{pH}L_A$ samples and was similar to that calculated for the $L_{pH}L_A$ combination ($p > 0.05$). When rates were compared among all treatments, values were higher under low pH conditions than under high pH treatment for all samples exposed to the H_A status ($p < 0.05$). However, at the medium NH_4^+ concentration, differences in responses were not significant ($p > 0.05$). When NH_4^+ levels were elevated, uptake rates were increased at each pH value (Table 2). In particular, the rate was approximately 41 times faster for $L_{pH}H_A$ than for $H_{pH}L_A$.

Growth rates, chlorophyll fluorescence, and C/N ratio

During the 14 days of observation, relative growth rates for *U. pertusa* ranged from 2.23 ± 0.13 to 4.27 ± 0.09% day^{-1} (Fig. 4), with values being lowest under $H_{pH}L_A$ conditions. The highest rate was associated with the $L_{pH}H_A$ treatment and was significantly different from rates calculated for the other

Table 3 Maximum values and extent of variation in pH in response to different culture treatment combinations over 8 h

	Maximum pH		Extent of variation	
	L_{pH} (low pH)	H_{pH} (high pH)	L_{pH} (low pH)	H_{pH} (high pH)
L_A (low NH_4^+)	10.11 ± 0.01a	10.12 ± 0.17a	2.61 ± 0.01a	2.12 ± 0.17b
M_A (medium NH_4^+)	10.14 ± 0.06a	10.14 ± 0.06a	2.64 ± 0.06a	2.14 ± 0.06b
H_A (high NH_4^+)	10.26 ± 0.07a	10.19 ± 0.01a	2.76 ± 0.07a	2.19 ± 0.01b

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

treatments ($p < 0.05$). At a high NH_4^+ level, growth was more rapid under low pH conditions than under high pH conditions ($p < 0.05$). By contrast, low and medium levels of NH_4^+ did not have a significant effect on growth rates ($p > 0.05$). At each pH level, relative growth rates increased as the NH_4^+ concentration rose (Table 2).

Photosynthetic efficiencies, as measured by chlorophyll fluorescence, ranged from 0.54 ± 0.04 ($H_{pH}L_A$) to 0.67 ± 0.06 ($L_{pH}H_A$) after 14 days (Fig. 5). Significant differences were detected between the $L_{pH}L_A$ and $L_{pH}H_A$ conditions (Fig. 5, Table 2). Although F_v/F_m values were greater under high NH_4^+ than under other conditions, they were similar when the low NH_4^+ and medium NH_4^+ treatments were compared.

Neither the pH conditions generated by the addition of CO_2 nor NH_4^+ concentrations affected the C/N ratios of *U. pertusa* (Table 2). Levels of C and N were 25.65 ± 0.65 to 27.26 ± 0.73% and 3.09 ± 0.04 to 3.15 ± 0.06%, respectively, and did not differ significantly among treatments ($p > 0.05$). The C/N ratio in tissues ranged from 8.24 ± 0.15 to 8.74 ± 0.22, but did not differ significantly among culture conditions (Table 4).

Discussion

The physiological responses of *Ulva pertusa* were influenced by pH and NH_4^+ status. This was demonstrated by the changes in pH, oxygen evolution, ammonium uptake, algal growth, chlorophyll fluorescence, and C/N ratio. The addition of CO_2 gas to the culture medium, which was used to mimic OA, altered pH values because of chemical reactions in the solution. When that gas was dissolved, carbonic acid formed and then partitioned into bicarbonate and hydrogen ions (Falkowski and Raven 2007). Through this process, the dissolved hydrogen ions caused pH to decrease while the DIC concentration increased in that solution. We noted that changes in pH values were greater under low pH conditions than under the ambient pH treatment due to photosynthetic activity. This was a result of greater DIC available for photosynthesis, which enhances pH levels (Zhang et al. 2012). In response to higher CO_2, many species down-regulate their CCM activity to save energy, thereby improving their rates of growth (Sarker et al. 2013).

Although our data showed that pH values under all of our culture conditions rose to almost 10.20 after 8 h, those differences were not significant. Zou (2014) has demonstrated that the pH compensation point of *Ulva prolifera* remains near

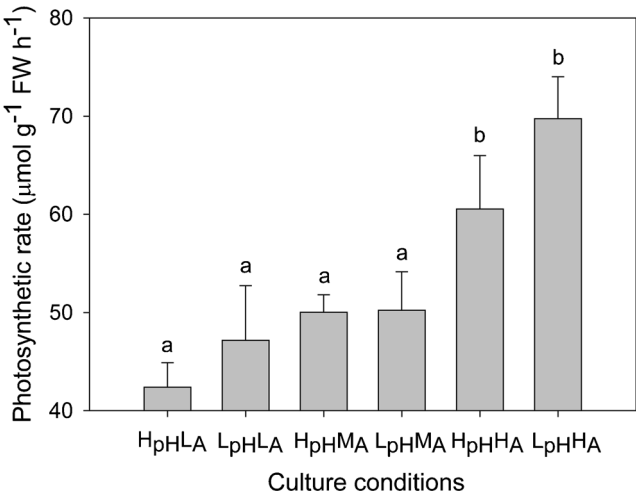

Fig. 2 Photosynthetic oxygen evolution rates (μmol O_2 g^{-1} FW h^{-1}) in response to different media culture conditions. Significant differences among conditions are indicated by different *letters* ($p < 0.05$). Data are means ± SD (n = 4)

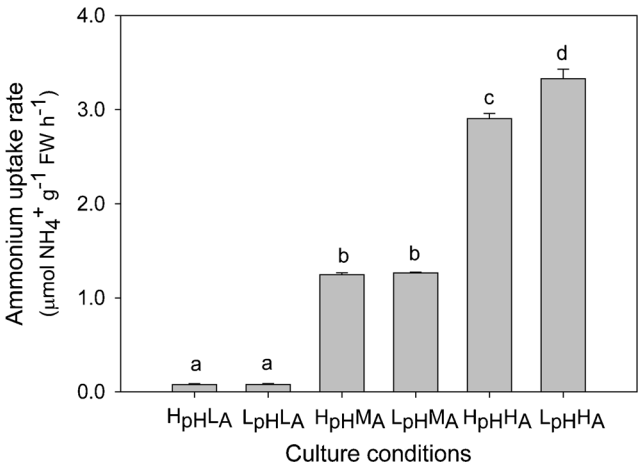

Fig. 3 Ammonium uptake rates (μmol NH_4^+ g^{-1} FW h^{-1}) by *Ulva pertusa* under different culture conditions. Significant differences among treatments are indicated by different *letters* ($p < 0.05$). Data are means ± SD (n = 4)

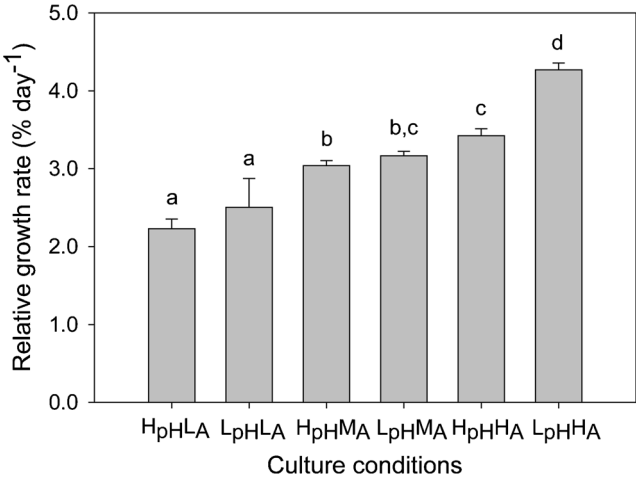

Fig. 4 Relative growth rates of *Ulva pertusa* under different CO_2 and ammonium levels. Significant differences among culture conditions are indicated by different *letters* ($p < 0.05$). Data are means ± SD ($n = 4$)

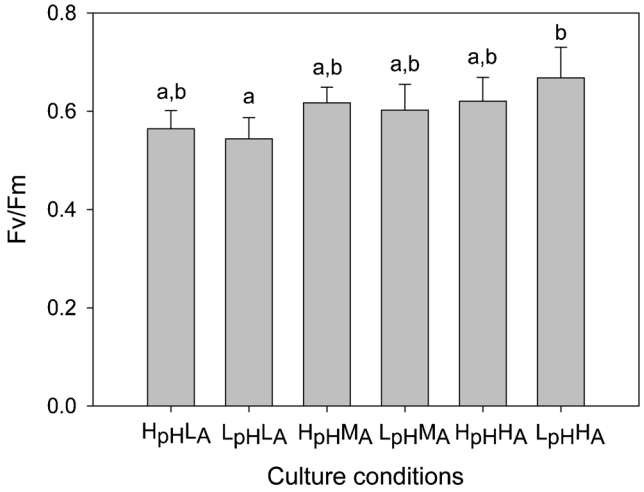

Fig. 5 Photosynthetic efficiency (F_v/F_m) of *Ulva pertusa* under different culture combinations. Significant differences among treatments are indicated by different *letters* ($p < 0.05$). Data are means ± SD ($n = 4$)

pH 10.40 under experimental conditions and pH is not affected by different culturing treatments because *U. prolifera* has a similar extract HCO_3^- capacity under a range of conditions. Maberly (1990) has reported that photosynthesis is limited at pH >9.0, which is contrary to our results. Under natural conditions, *Ulva* spp. utilize two means for taking up HCO_3^- when pH levels are high. The first mechanism involves external carbonic anhydrase, which converts, through dehydration, HCO_3^- to CO_2 that is then transported into the cells (Beer and Israel 1990; Axelsson et al. 1995; Larsson et al. 1997; Björk et al. 2004). The second possible mechanism is direct uptake of HCO_3^- via anion exchange protein under experimental conditions (Axelsson et al. 1995; Larsson et al. 1997; Björk et al. 2004). We suspect that *U. pertusa* uses both mechanisms for taking up HCO_3^- under either low- or high-pH conditions in the laboratory (i.e., beaker cultivation).

We found that photosynthetic oxygen evolution rates for *U. pertusa* were influenced by elevated nutrient concentrations. Those rates improved under both low and high pH when NH_4^+ levels increased. At higher NH_4^+, the difference in relative rates was significant between low NH_4^+ and medium NH_4^+ conditions but not between the $L_{pH}H_A$ and $H_{pH}H_A$ treatments. These results demonstrated that oxygen evolution by *U. pertusa* is more strongly modulated by a rise in NH_4^+ concentrations than by pH levels.

The greater rates of NH_4^+ uptake by *U. pertusa* in response to elevated CO_2 and NH_4^+ levels or NH_4^+ alone noted here are similar to those reported for *Porphyra leucosticte*, *Ulva rigida*, *Sargassum fusiforme*, *Gracilaria lemaneiformis*, *U. prolifera*, *Ulva linza*, and *Pyropia haitanensis* (Mercado et al. 1999; Gordillo et al. 2001; Zou 2005; Xu et al. 2010; Luo et al. 2012; Xu and Gao 2012; Chen et al. 2016). For example, higher N concentrations lead to faster uptake by *Ulva* spp. (Luo et al. 2012). Elevated CO_2 levels also enhance the uptake of N (Gordillo et al. 2001; Zou 2005; Liu and Zou 2015). When more nutrients are available, *Ulva* spp. can take up N more efficiently (Björnsäter and Wheeler 1990; Runcie et al. 2003; Pérez-Mayorga et al. 2011). The distinct morphological characteristics of *Ulva* spp. facilitate the opportunistic uptake of nutrients, which can then increase growth rates when NH_4^+ concentrations are higher (Duke et al. 1987; Arévalo et al. 2007). We also found that uptake rates were greatest in response to the interactive effect of $L_{pH}H_A$ treatment. Therefore, future investigations should focus on aspects of

Table 4 Tissue C, N (% in DW), and C/N molar ratio of *Ulva pertusa* under different culture conditions

	C (%)	N (%)	C/N molar ratio
$L_{pH}L_A$ (low pH/low NH_4^+)	27.26 ± 0.73a	3.15 ± 0.06a	8.65 ± 0.33a
$H_{pH}L_A$ (high pH/low NH_4^+)	26.98 ± 1.02a	3.09 ± 0.04a	8.74 ± 0.22a
$L_{pH}M_A$ (low pH/medium NH_4^+)	26.44 ± 0.59a	3.15 ± 0.04a	8.40 ± 0.21a
$H_{pH}M_A$ (high pH/medium NH_4^+)	26.23 ± 0.98a	3.13 ± 0.05a	8.37 ± 0.29a
$L_{pH}H_A$ (low pH/high NH_4^+)	25.65 ± 0.65a	3.11 ± 0.04a	8.24 ± 0.15a
$H_{pH}H_A$ (high pH/high NH_4^+)	25.87 ± 0.78a	3.11 ± 0.02a	8.32 ± 0.20a

Data are means ± SD ($n = 4$). Values not followed by same letter are significantly different at $p < 0.05$

metabolism, e.g., algal growth and nutrient uptake, in combination with CO_2, NH_4^+, and other environmental factors such as light intensity and temperature (Suárez-Álvarez et al. 2012). Our results indicated that relative growth rates of *U. pertusa* were faster when either nutrient levels alone or in combination with CO_2 were increased. Both factors enhance seaweed growth and biomass accumulations in *U. prolifera*, *U. linza*, *Hypnea spinella*, *Chondrus crispus*, and *P. haitanensis* (Luo et al. 2012; Suárez-Álvarez et al. 2012; Xu and Gao 2012; Sarker et al. 2013; Chen et al. 2016). In our experiments, algal growth was maximized under $L_{pH}H_A$ conditions and performance was significantly different when compared with results from the other treatments. Young and Gobler (2016) have also reported that the growth of *Ulva* spp. is promoted by elevated CO_2 and nutrient concentrations. Because *U. pertusa* utilizes both CO_2 and NH_4^+ for photosynthesis, that process can possibly be improved under OA and eutrophication conditions. In fact, such a scenario has been described with *G. lemaneiformis* and *P. haitanensis* (Xu et al. 2010; Chen et al. 2016). Therefore, we conclude that the interaction between CO_2 and nutrient levels can have a dramatic impact on the growth of *U. pertusa*.

In addition to some changes in pH values, ammonium uptake rates, and relative growth rates, the photosynthetic efficiency (F_v/F_m) of *U. pertusa* was significantly affected by NH_4^+ concentrations. A previous study has found that photosynthetic efficiency is improved when nitrogen is more readily available (Zou and Gao 2014). Dawes and Koch (1990) have also shown that increased N concentrations can enhance chlorophyll fluorescence in algae. This is in contrast to a previous report that chlorophyll fluorescence is greater under higher CO_2 concentrations (Chen et al. 2015). However, Liu et al. (2012) have found that elevated CO_2 induces photochemical stress in *U. prolifera* under high-light conditions. Our results indicated that *U. pertusa* was not subjected to such stress because the experiments involved less intense lighting.

Regardless of treatment, the C/N ratio of *U. pertusa* was not significantly affected by elevated CO_2 or NH_4^+ concentrations. Similar findings have been described for *Porphyra leucosticta* and *Hypnea spinella* (Mercado et al. 1999; Suárez-Álvarez et al. 2012). However, we calculated a C/N ratio of approximately 8:1, which is lower than the ratio of 10:1 that generally occurs when nitrogen supplies are abundant (Gómez-Pinchetti et al. 1998; Suárez-Álvarez et al. 2012). Chen and Johns (1991) have suggested that the C/N ratio is a good tool for assessing the physiological status of macroalgae. In fact, under our culture conditions, samples were able to maintain a good status throughout the experimental period.

Although *U. pertusa* is currently regarded as a harmful species in several coastal areas, our findings indicated that it can also provide a mitigating solution to problems associated with acidification and eutrophication because of its capacity to take up nutrients and DIC. Nevertheless, this study focused on short-term responses by *U. pertusa* and was conducted only on a small experimental scale. Therefore, further investigations should include long-term and large-scale examinations, such as mesocosm and field studies, so that researchers can obtain more practical information as they develop new strategies to deal with OA and eutrophication.

In summary, our data demonstrated that the physiological activities of *U. pertusa* are positively affected by OA and eutrophication. Increases in either CO_2 levels or NH_4^+ concentrations had a strong impact on variations in pH, photosynthetic oxygen evolution rates, and chlorophyll fluorescence. In particular, the combination of elevated CO_2 and NH_4^+ had the greatest influence on NH_4^+ uptake and algal growth. By comparison, rises in levels of CO_2 and NH_4^+ did not affect the C/N ratio of this species.

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